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FURTHER STUDIES ON PENICILLIUM INJURY TO CORN[1]

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INTRODUCTION

It is generally recognized that, although considerable study has been given to diseases of corn in recent years, there still remains more or less uncertainty concerning the symptoms, causal organisms, and pathological effects of the various blights and rots encountered in field studies and on the germinator. During the winter and spring of 1927 an unusual amount of disease was found on the inbred lines of corn germinated in the course of breeding experiments at the University of Wisconsin. Some injuries were recognized as due to known pathogenes. Others seemingly were caused by a species of Penicillium. On certain seedlings, tufts of Penicillium hyphae were visible at the hilum end of the kernel and along the surface of the embryo within the broken pericarp. Whether the fungus was located on the coleorhiza or along the embryo toward the base of the mesocotyl, the result of its presence apparently was a gradual retardation of growth followed not infrequently by the death of the young seedling. When platings had confirmed the diagnosis and preliminary inoculations in the greenhouse had given positive results, the present investigation was begun in order to differentiate this disease from other seedling blights of corn and to study it in more detail.

A transfer from the culture that was the source of the inoculum used in these experiments was examined by Charles Thom (*17, p. 82*),[3] who considered it to be "close to, if not identical with, *Penicillium oxalicum* Currie and Thom."

Inoculation experiments have been conducted in the greenhouse and in the field at Madison, Wis., and in the field at Bloomington, Ill. Cultural and histological studies of fungus and host have been made n the laboratories at the University of Wisconsin (*9*).

REVIEW OF LITERATURE

A number of investigators have reported finding *Penicillium* spp. in corn or corn products.

Manns and Adams (*12, p. 521*), in their studies of the internal fungi of seed corn, note the presence of *Aspergillus niger* and *Penicillium* spp.

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[3] Reference is made by number (italic) to Literature Cited, p. 790.

They consider these organisms of minor importance in their problem and suggest that "they have become established because of unfortunate conditions under which the corn has been exposed and stored." They add: "These types of fungi on the germinator may also retard or in some cases inhibit germination."

Koehler (*11, p. 469*) observes that "isolations from seedlings grown from susceptible seed in soil at 16° C. showed that species of Mucor, Penicillium, and Fusarium were the predominating organisms associated with scutellum rot."

Melchers and Johnston (*13*) found *Fusarium moniliforme* to be by far the most common fungus on the ears which they tested on the germinator. As regards other fungi present, they report that "Penicillium, Rhizopus, and Aspergillus are much more common than Diplodia and Gibberella."

In the above papers the species of Penicillium in question were apparently undetermined. Possibly more than one species were present.

According to Currie and Thom (*3*), *Penicillium oxalicum* has been obtained from moldy corn in Kansas and from corn meal in Connecticut, Maryland, Virginia, and Illinois.

Thom and LeFevre (*18, p. 185*) remark that "several strains of Penicillium are found in meal cultures. * * * *P. oxalicum* Thom and Currie is found in many samples of meal, but rarely in miscellaneous culture work."

In 1928 and 1929, the senior writer published (*8, 9*) the results of preliminary studies of *Penicillium oxalicum* on corn seedlings.

Thom in 1930, further describes *Penicillium oxalicum* and notes as follows the cultural characteristics (*17, pp. 247, 248*) of the strain on corn studied by the writers:

> A soil inhabiting species producing oxalic acid rapidly in sugar media. Colonies ivy-green velvety, broadly spreading, with reverse pale yellow, and agar uncolored or only slightly colored; conidiophores up to 200 μ by 3.3 to 5.4 μ, producing a verticil of 2 to 3 metulae, verticils of closely parallel sterigmata 10 to 14 by 2.5 to 3.5 μ; conidia cylindrical then elliptical, uneven in size from 3 by 2 μ when first formed to a maximum of 5 by 3.5 μ when ripe and becoming subglobose 7 to 10 μ in diameter in germination, in long chains packed in close columns. In favorable media producing great masses of conidia which break off readily.
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> A variation of this species (no. 4913) received from Helen Johann at Madison, Wis., is discussed by her as injuring corn seedlings (Phytopathology 18: 239–242, 1928). Notes on this strain are added as showing a range of cultural characters in the species:
>
> Colonies on Czapek's solution agar, broadly spreading, azonate, or showing a trace of zonation when viewed from below, velvety, in color a dark dull green shade nea rsage green to slate olive [4] * * * but with a slightly bluish tinge near the margin; reverse colorless or slightly yellowed; odor, none; drops not seen; conidiophore about 100 to 200 by 3 μ, smooth; penicillus either monoverticillate or mostly with one branch or metula (sometimes two), usually appressed and with the conidial chains forming one or two columns often 500 μ or longer and commonly 10 to 15 μ in diameter, forming tangled masses over the whole colony which break off when struck or topped; metulae about 20 by 3 μ, in groups of 2 to 3; sterigmata up to 12 to 14 μ by about 3 μ, taper pointed; conidia elliptical, about 5 to 6 by 3 to 4 μ mostly 5 to 5.5 by 3 to 3.5 μ.

Shapovalov (*15, p. 38*) also has mentioned this species in connection with a rot of potato tubers. He says:

> On the underground portions of the growing potato plants this fungus, when artificially inoculated without wounding, produced dark depressed lesions in some cases and only a mild infection in others.

[4] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. Plate 47. Washington, D. C. 1912.

GENERAL SYMPTOMS OF PENICILLIUM INJURY

Symptoms of seedling injury caused by *Penicillium oxalicum* are manifest in a yellowing of the leaves, a retardation in the growth of the plant, and, in cases of severe infection, more or less complete drying of the leaves. In the last case, death usually occurs before the sixth leaf has unfolded. The dead plants, both in the greenhouse and in the field, remain upright. (Fig. 17.) Such plants, as well as others less severely injured, show scarcely any rotting of the mesocotyls or root systems. This is in striking contrast with the conspicuous rotting of these parts by *Gibberella saubinetii* and *Diplodia zeae*.

In cases of mild seedling infection, the yellow leaves may recover their normal green color within two weeks. As the season advances, the effect of the mild seedling infection during the seedling stage may be shown in retarded growth and lessened yield. However, these symptoms by themselves can not be distinguished from those resulting from other causes.

In general, plants from seed inoculated with *Penicillium oxalicum* were from two to five days later in tasseling and silking than were those of the same strains grown in adjacent plots from uninoculated seed. In the fall, the plants from inoculated seed were correspondingly later in attaining normal maturity.

EARLIER GREENHOUSE EXPERIMENTS

Infection experiments were conducted in the Wisconsin soil-temperature tanks in the greenhouse at Madison, Wis., during the winter of 1927–28. The field soil used was autoclaved 3 times at 48-hour intervals at a pressure of 15 pounds for 3 hours. It was then screened, made up to a good growing moisture, and, after the seeds had been planted, it was covered to a depth of one-half to 1 inch with a layer of ground cork in order to retard evaporation from the surface. The seed had been inoculated by immersion in a heavy spore suspension for approximately half an hour. It was found to be a comparatively simple matter to make the spore suspension if water at a temperature of 40° to 45° C. was used. With cold water, it was difficult to wet the surface of the Penicillium spores even after a long and thorough shaking.

TEMPERATURE RELATIONS

Repeated experiments carried on in the Wisconsin soil-temperature tanks in the greenhouse indicate that the maximum seedling injury occurs near 28° C. (*8*). (Fig. 1.) At that temperature a large percentage of the plants were killed in the second-leaf or third-leaf stage. At 24° reduction in stand was slightly less than at 28°; at 20° the plants became more or less yellow, but a fair percentage recovered. The minimum injury occurred at 16°, a temperature at which there is considerable reduction in stand following inoculation with either *Gibberella saubinetii* (*4*) or *Pythium arrhenomanes* (*10*).

The various inbred strains of corn did not prove equally susceptible to Penicillium injury under greenhouse conditions. The inbred strain B–R–10, which normally makes a rapid growth and soon attains considerable height, showed a greater reduction in stand and in height of plants than did strain A–1237 under similar conditions. (Fig. 2.)

SYMPTOMS FOLLOWING INOCULATION

Seedlings from seed inoculated with a spore suspension of Penicillium at time of planting in autoclaved soil emerged in a seemingly normal condition.

FIGURE 1.—Corn seedlings of inbred strain B–R–10, 12 days old, grown in the greenhouse at a temperature of 28° C. from (A) uninoculated seed (one can) and from (B) seed inoculated (three cans) with *Penicillium oxalicum* at time of planting

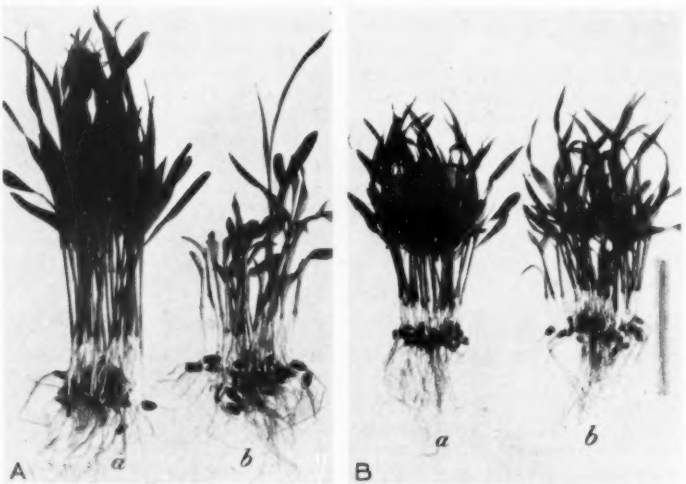

FIGURE 2.—Seedlings of two inbred strains of dent corn grown at 24° C. from uninoculated seed and from seed inoculated with *Penicillium oxalicum* at time of planting. Each group comprises all the plants produced by 40 kernels. A, Inbred strain B–R–10; *a*, from uninoculated seed; *b*, from inoculated seed. B, Inbred strain A–1237; *a*, from uninoculated seed; *b*, from inoculated seed

The first symptom of Penicillium injury, a reed-yellow to olive-yellow [5] color of the leaves, appeared within a few days after the first leaf had ruptured the coleoptile. In the most severe cases the first

[5] RIDGWAY, R. Op. cit.

leaf became chlorotic and the second leaf seldom unfolded. More often, symptoms of injury did not appear so early. In such cases the first leaf was a normal green color. The lower half of the second leaf, however, soon became yellow. The chlorotic area in some instances was confined to the basal half and in others it included the entire leaf. Before the third leaf unfolded, in cases of severe infection, the tips and margins of the leaves had become dry and brittle. (Pl. 1.) Little change in color accompanied this drying. The seedlings remained upright for a time after the leaves had become brittle and had ceased to function. No extensive rot of the roots or mesocotyls was observed in these experiments.

When infections were severe enough to kill the seedling, death usually took place in the second-leaf or third-leaf stage, although occasionally it was delayed until the fifth leaf had unfolded. When less severe infection occurred, the leaves showed more or less yellowing for perhaps a week or 10 days, then recovered their normal color. (Pl. 2.) During this period, however, growth was retarded, and this retardation continued indefinitely, as shown in the yields of surviving plants in the inoculated field series.

As will be shown later, *Penicillium oxalicum* is essentially saprophytic in its habit. Consequently its location in the tissues and the symptoms associated with its presence are less clearly defined than in the wilt diseases of other plants caused by Fusaria, in which the hyphae are largely confined to the vessels.

FIELD EXPERIMENTS

Field experiments were conducted in 1928 on the farm at the University of Wisconsin, Madison, Wis., and in 1928 and 1929 at Bloomington, Ill.

EXPERIMENTS AT MADISON, WIS., IN 1928

The plot at Madison was planted with seed (1) of inbred strains developed at Bloomington, Ill., and grown at Bloomington and at Madison, and (2) of inbred strains developed cooperatively by the departments of plant pathology, genetics, and agronomy of the University of Wisconsin and grown at Madison.

The seed of the Illinois strain B–R–10, grown at Madison in 1927, was not so well matured as that grown the same year at Bloomington. Consequently, as shown in Table 1, the stand was very greatly reduced in both the inoculated and uninoculated hills where this seed was planted, in contrast with the stand from seed of the same strain grown and more fully matured at Bloomington.

As shown in Tables 1 and 2, the Wisconsin strains as a group showed a somewhat greater reduction in stand following inoculation than did the Illinois strains. For the Illinois strains, the average percentage reduction in stand following inoculation with *Penicillium oxalicum* was 33.1 per cent. For the Wisconsin strains, it was 42.6 per cent. In only one case in each group did the stand of the inoculated series equal or exceed that of the uninoculated. In each group a few strains showed markedly less reduction in stand than the others.

TABLE 1.—*Field stand of Illinois inbred strains of yellow dent corn grown at Madison, Wis., in 1928, from uninoculated seed and from seed inoculated with Penicillium oxalicum*

Strain No.	Seed grown in 1927 at—	Kernels planted	Stand from seed—		Percentage increase (+) or decrease (−) in stand following inoculation
			Uninoculated	Inoculated	
		Number	*Per cent*	*Per cent*	*Per cent*
A–9	Madison, Wis	20	45.0	10.0	−77.8
A–9	Bloomington, Ill	20	60.0	35.0	−41.7
B–R–10	Madison	30	63.3	33.3	−47.4
B–R–10	do	20	60.0	5.0	−91.7
B–R–10	Bloomington	30	90.0	86.7	−3.7
A–48	do	30	43.3	33.6	−22.4
A–956	do	20	75.0	40.0	−46.7
A–1237	do	20	100.0	85.0	−15.0
L–111	do	30	80.0	83.3	+4.1
Average			68.5	45.8	[a]−33.1
Odds					334 : 1

[a] Based on difference in average stands.

TABLE 2.—*Field stand of 18 Wisconsin inbred strains of dent corn grown at Madison, Wis., in 1928, from uninoculated seed and from seed inoculated with Penicillium oxalicum*

Strain No.	Kernels planted	Stand from seed—		Percentage increase (+) or decrease (−) in stand following inoculation	Strain No.	Kernels planted	Stand from seed—		Percentage increase (+) or decrease (−) in stand following inoculation
		Uninoculated	Inoculated				Uninoculated	Inoculated	
	Number	*Per cent*	*Per cent*	*Per cent*		*Number*	*Per cent*	*Per cent*	*Per cent*
640–12	20	80.0	25.0	−68.8	848–10	30	83.3	76.7	−7.9
652–8	30	73.3	56.7	−22.6	859–11	30	80.0	56.7	−29.1
654–9	30	73.3	40.0	−45.4	859–20	30	73.3	30.0	−59.1
659–8	30	86.7	30.0	−65.4	871–16	30	70.0	20.0	−71.4
782–15	30	80.0	53.3	−33.4	872–39	20	90.0	0.0	−100.0
797–11	30	63.3	46.7	−26.2	886–19	20	50.0	15.0	−70.0
801–14	20	85.0	55.0	−35.3	920–8–g	20	90.0	70.0	−22.2
818–9	30	70.0	60.0	−14.3					
824–6	30	76.7	93.3	+21.6	Average		75.3	43.2	[a]−42.6
839–3	30	70.0	13.3	−81.0	Odds				>9,999 : 1
845–5	30	60.0	36.7	−38.8					

[a] Based on difference in average stands.

EXPERIMENTS AT BLOOMINGTON, ILL.

WEATHER CONDITIONS IN 1928

The United States Weather Bureau (*19, v. 33, p. 17*), summarizing weather conditions in Illinois for May, 1928, says in part:

> May was a very pleasant month, with excessive sunshine, low humidity, and rather light rainfall. In only three previous years [39 years' record] has there been less cloudiness. During the first half of the month the rainfall was deficient over most of the State, and the last decade was dry throughout much of the north and central areas.

The mean temperature for Bloomington was 63.2° F., 0.5° above normal. The highest temperature was 91° on May 10 (the day after the corn was planted). Figure 3 shows the daily mean temperature

and the daily rainfall from May 1 to June 15 at Bloomington. The rainfall for May amounted to 2.42 inches, 1.91 inches less than normal. The prevailing direction of the winds was southwest.

June was relatively cool, the mean temperature of 66.6° F. being 5.4° below normal. The total precipitation of 5.17 inches was 1.28 inches above normal. For this month also the prevailing direction of the winds was southwest.

Results of Inoculations in 1928

During the first days of June the seedlings from the seed inoculated with Penicillium showed such a decided yellowing that in several cases the inoculated plots were clearly marked by their uniformly light yellow-green color. By June 10, blighting had almost wiped

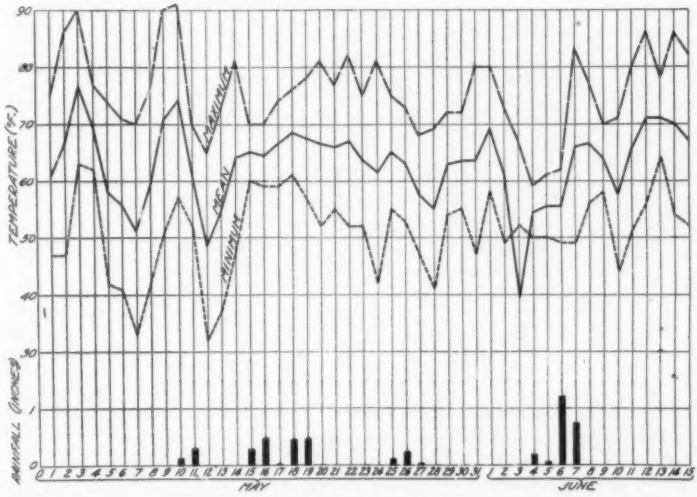

FIGURE 3.—Daily air temperatures and rainfall at Bloomington, Ill., May 1 to June 15, 1928

out some strains. In other strains the plants were regaining their normal color.

The data on field stands and on plant and acre yields, from both the uninoculated and the inoculated series, are given in Table 3. Stands and acre yields were reduced in all the inoculated plots as compared with those in the uninoculated controls. In the inbred strains, the reduction in stand ranged from 14.1 per cent for B–R–10 (fig. 4) to 100 per cent for A–956 (fig. 5). Notwithstanding the reduced stands following inoculation, the mean yield per plant in each of the inbred strains was lower for plants grown from inoculated seed than for plants grown from uninoculated seed. In contrast with this, in F_1 250, following inoculation, although the stand was reduced 12.3 per cent, the color of the remaining young plants was normal green, and the yield per plant was slightly higher from the plants grown from inoculated seed than from those grown from uninoculated seed. This would indicate that F_1 250 was more resistant to the attacks of Peni-

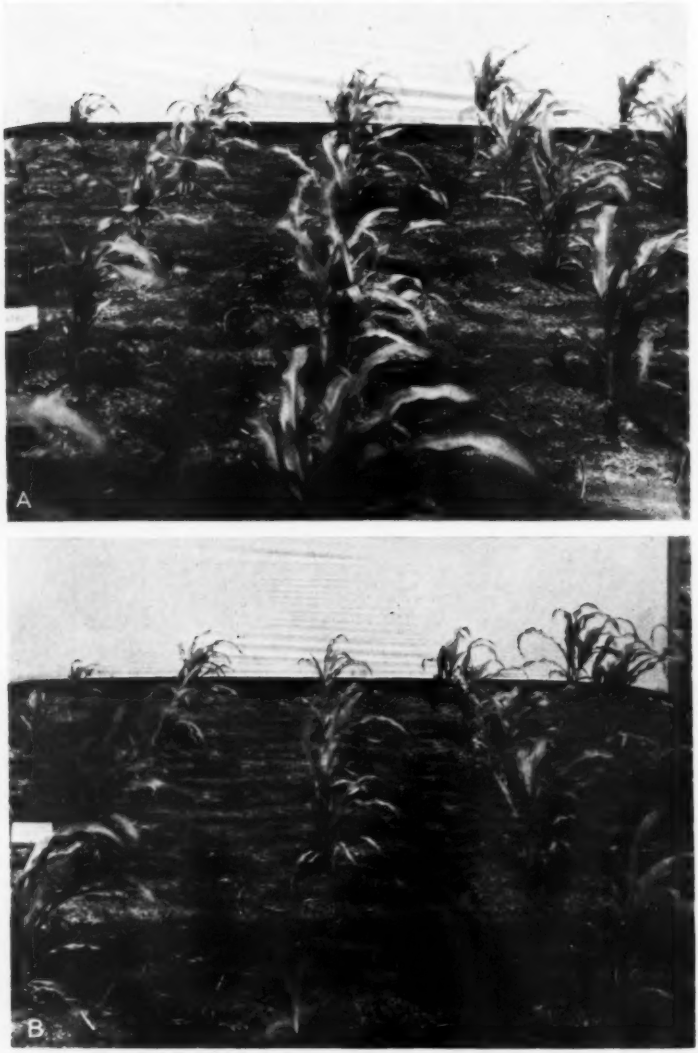

FIGURE 4.—Corn plants of inbred strain B–R–10, grown at Bloomington, Ill., in 1928, (A) from uninoculated seed, and (B) from seed inoculated with *Penicillium oxalicum* at time of planting

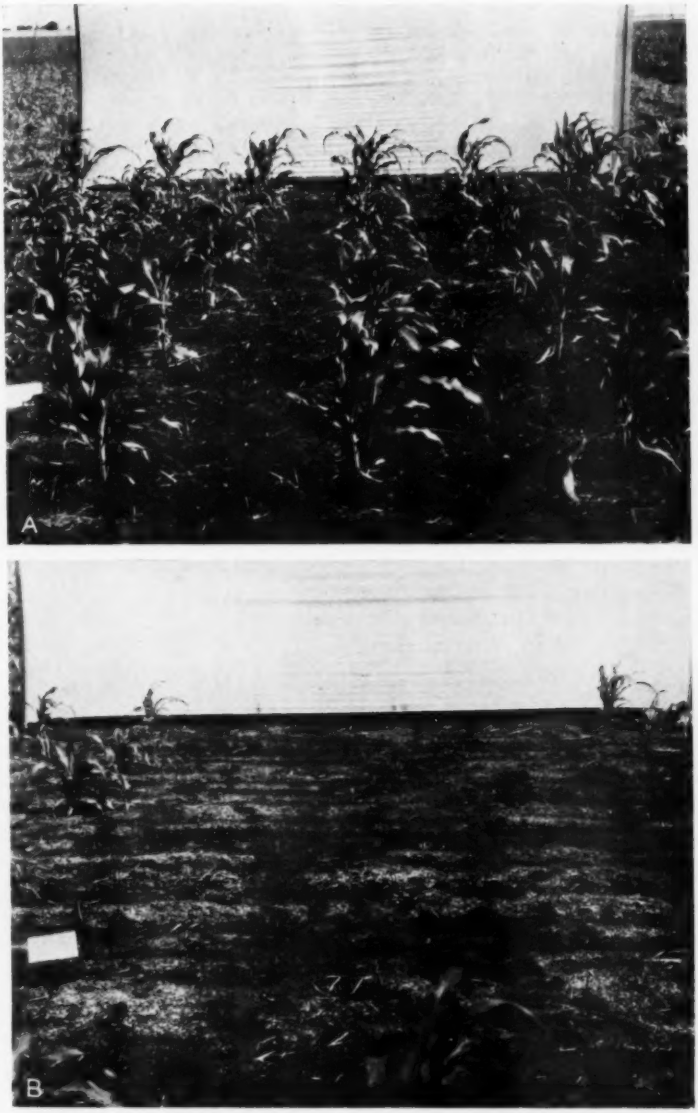

FIGURE 5.—Plants of a strain of yellow dent corn (A-956) susceptible to *Penicillium oxalicum*, grown at Bloomington, Ill., 1928, (A) from uninoculated seed and (B) from seed inoculated with *P. oxalicum* at time of planting

TABLE 3.—*Field stand and mean yield per plant and per acre of seven inbreds and one first-generation cross of corn varieties grown from uninoculated seed and from seed inoculated with a spore suspension of Penicillium oxalicum at planting time, near Bloomington, Ill., May 9, 1928*

Strain	Replications	Field stand from seed—		Percentage decrease in stand following inoculation	Odds	Plant yield from seed—		Percentage Increase (+) or decrease (−) in plant yield following inoculation	Odds	Acre yield from seed—		Percentage Decrease in acre yield following inoculation	Odds
		Uninoculated	Inoculated			Uninoculated	Inoculated			Uninoculated	Inoculated		
	Number	*Per cent*	*Per cent*	*Per cent*		*Pound*	*Pound*	*Pound*		*Bushels*	*Bushels*	*Bushels*	
F_1 250 (A–1237–× B–R–10)	15	92.0	80.7	12.3	9,999:1	0.689	0.715	+0.026	85:1	111.2	101.1	10.1	9,999:1
Inbred:													
B–R–10	4	98.9	85.0	14.1	89:1	.355	.297	−.058	20:1	61.5	44.4	17.1	36:1
A–1237	4	84.5	44.4	47.5	999:1	.241	.154	−.087	50:1	37.7	13.2	24.5	157:1
A–48	3	67.4	2.9	95.7	210:1	.265	0	−.265	2,749:1	33.7	0	33.7	143:1
A–9	4	85.0	29.5	65.3	1,484:1	.235	.224	−.011	39:1	37.6	12.3	25.3	839:1
A–3	4	46.7	1.7	96.4	666:1	.232	.075	−.157	13:1	20.5	.9	19.6	310:1
Leaming	4	85.0	68.9	18.9	6,749:1	.353	.332	−.021	10:1	56.2	42.4	13.8	111:1
A–956	4	71.1	0	100.0	1,826:1	.292	0	−.292	7,169:1	39.2	0	39.2	942:1

cillium than were either of its parent inbreds, A–1237 and B–R–10, or any of the other inbred strains included in this experiment. The reasons why the plant yields of F_1 250 were slightly higher in the inoculated than in the uninoculated series that were doubtless (1) the reduced stand in the inoculated series lessened competition for the individual plants, and (2) the remaining plants in the inoculated series were highly resistant to the attacks of the Penicillium and, being able to take advantage of the lessened plant competition, produced higher yields per plant.

In all the strains, with the exception of A–48, A–3, and A–956, there were plants that appeared to be highly resistant. The question naturally arose as to whether this result was due primarily to resistance or to lack of uniformity in the inoculation. This point was kept in

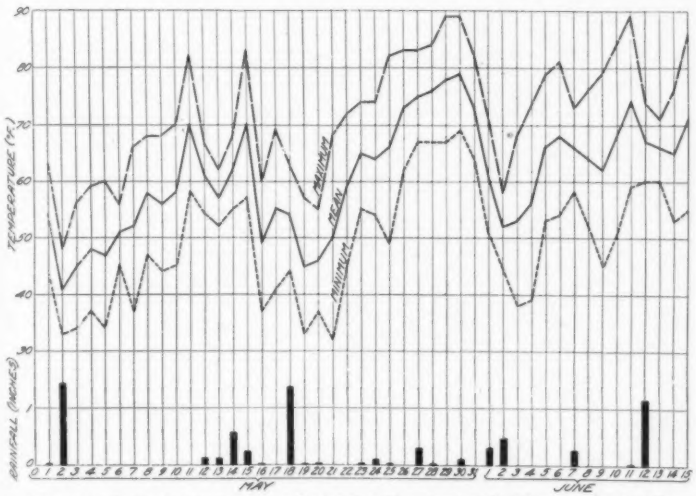

FIGURE 6.—Daily air temperatures and rainfall at Bloomington, Ill., May 1 to June 15, 1929

mind in subsequent greenhouse experiments, and special care was taken to insure adequate and uniform inoculations.

WEATHER CONDITIONS IN 1929

In contrast with conditions in 1928 the spring of 1929 was generally wet and cool. Figure 6 shows the daily mean temperature and the daily rainfall at Bloomington (*19*) from May 1 to June 15, 1929.

RESULTS OF INOCULATIONS IN 1929

To determine relative resistance to Penicillium, different hybrids were grown from uninoculated and inoculated seed on unfertilized and fertilized soil. The results are given in Table 4. Similar limited data also were obtained on numerous inbred strains. In another experiment, conducted to determine the effect of seed condition on susceptibility to injury by Penicillium, uninoculated seeds and inoculated seeds were planted. The results are given in Tables 5 and 6.

TABLE 4.—*Field stand and mean yield per plant and per acre of three groups of corn hybrids having (1) both inbred parents comparatively resistant to Penicillium in 1928 and 1929, (2) one inbred parent resistant and the other susceptible to Penicillium, (3) both inbred parents susceptible to Penicillium, grown from uninoculated seed and from seed inoculated at planting time with P. oxalicum and planted May 11, 1929, near Bloomington, Ill., on unfertilized soil and on soil fertilized by a broadcast application of a 5–15–5 fertilizer[a] at the rate of 600 pounds per acre*

Soil and group	Replications (45 kernels in each)	Field stand from seed—		Percentage increase (+) or decrease (−) in stand following inoculation	Odds	Plant yield from seed—		Percentage increase (+) or decrease (−) in plant yield following inoculation	Odds	Acre yield from seed—		Decrease in acre yield following inoculation		Odds
		Uninoculated	Inoculated			Uninoculated	Inoculated			Uninoculated	Inoculated			
Unfertilized soil:	*Number*	*Per cent*	*Per cent*	*Per cent*		*Pound*	*Pound*	*Per cent*		*Bush.*	*Bush.*	*Bush.*	*Per cent*	
1.—Both parent inbreds resistant to Penicillium	9	69.3	73.5	+6.1	6:1	0.723	0.673	−6.9	37:1	75.5	73.7	1.8	2.4	2:1
2.—One parent inbred resistant and the other susceptible to Penicillium	11	67.3	60.0	−10.8	9:1	.659	.648	−1.7	2:1	66.2	60.1	6.1	9.2	5:1
3.—Both parent inbreds susceptible to Penicillium	7	77.3	70.7	−8.5	6:1	.505	.481	−4.8	20:1	61.4	52.7	8.7	14.2	155:1
Average	27	71.3	68.1	[b]−4.5	3:1	.629	.601	−4.5	34:1	67.7	62.2	5.5	[c]8.1	49:1
Fertilized soil:														
1.—Both parent inbreds resistant to Penicillium	9	81.3	77.3	−4.9	5:1	.717	.719	+0.3	4:1	87.9	83.6	4.3	4.9	7:1
2.—One parent inbred resistant and the other susceptible to Penicillium	11	73.3	66.7	−9.0	>9,999:1	.684	.664	−2.9	2:1	75.6	63.5	12.1	16.0	382:1
3.—Both parent inbreds susceptible to Penicillium	7	78.0	66.0	−15.4	83:1	.509	.452	−3.3	3:1	65.3	54.9	10.4	15.9	83:1
Average	27	77.5	70.0	[b]−9.7	>9,999:1	.637	.625	−1.9	3:1	76.3	67.3	9.0	[c]11.8	>9,999:1

[a] Nitrogen 5 per cent, phosphoric acid 15 per cent, and potash 5 per cent. [b] Based on difference in average stands. [c] Based on difference in average acre yields.

TABLE 5.—*Field stand[a] and yield per acre[a] of yellow dent corn from crossbred, fully viable, nearly disease-free seed produced in 1928 on plants of several 8-year to 12-year inbreds of two groups (Group 1, not killed by cold in September, 1928, and Group 2, killed during same period), some seed from both groups uninoculated and some inoculated with Penicillium oxalicum, and planted near Bloomington, Ill., May 11, 1929*

Group and reaction to cold in September, 1928	Field stand from uninoculated seed	Percentage decrease in field stand following inoculation	Acre yield from uninoculated seed	Decrease in acre yield following inoculation	
	Per cent	*Per cent*	*Bushels*	*Bushels*	*Odds*
1.—Not killed	90.0	3.0	88.1	3.6	6:1
2.—Killed	84.0	14.3	85.8	9.7	395:1

[a] Average of 10 replications; 45 kernels planted in each replication; yields corrected to 14 per cent moisture.

TABLE 6.—*Field stand[a] and mean yield per plant of inbred strain A–1237 of yellow dent corn grown from fully viable, nearly disease-free seed selected in 1928 from two groups in adjacent plots (Group 1, planted May 5, 1928, the seed of which matured before freezing, and Group 2, planted May 15, 1928, the seed of which was not fully matured before freezing), some seed from both groups uninoculated and some inoculated with Penicillium oxalicum, and planted near Bloomington, Ill., May 11, 1929*

Group	Field stand from uninoculated seed	Percentage decrease in stand following inoculation	Odds	Mean yield per plant from uninoculated seed	Percentage decrease in plant yield following inoculation	Odds
	Per cent	*Per cent*		*Pound*	*Per cent*	
1[b]	79.1	0		0.306		
2[c]	77.3	25.2	344:1	.211	31.0	336:1

[a] Average of 10 replications; 45 kernels planted in each replication.
[b] Seed matured before freezing.
[c] Seed not fully matured before freezing.

In Table 4 the data on the hybrids, both on unfertilized and fertilized soil, are assembled in three groups according to the reaction of the parent inbred strains to Penicillium inoculation. It will be noted that, on the basis of field stands and acre yields, the hybrids from Penicillium-resistant parents were most resistant, those from Penicillium-susceptible parents were most susceptible, and those with one parent resistant and the other susceptible either were intermediate or approached the reaction of the susceptible. It is of special interest to note that the average reductions of both stands and acre yields were greater on the fertilized soil than on the unfertilized soil. The average reductions in plant yields, however, were greater on the unfertilized soil than on the fertilized soil.

The results given in Tables 5 and 6 indicate that the degree of proper maturation of the seed on the mother plants may have an important influence on the relative resistance to Penicillium injury of the plants grown from such seed. In Table 5 the reductions in field stands and yields following inoculation were much greater in the plots planted with seed from plants killed by cold before maturity than in those planted with seed from plants not killed by cold before maturity. In Table 6 the reduction in field stand following inoculation was

markedly greater in the plots planted with seed from plants injured by cold before maturity. It is of interest to note that the mean plant yield from the uninoculated plots planted with Group 2 seed is significantly lower than the mean plant yield from uninoculated plots planted with Group 1 seed.

FIGURE 7.—Corn plants of inbred strain A–1237 grown at Bloomington, Ill., 1928, (A) from uninoculated seed and (B) from seed inoculated with *Penicillium oxalicum* at time of planting. A few seemingly resistant plants remain

MORE RECENT GREENHOUSE EXPERIMENTS

Additional inoculation experiments were conducted in the greenhouse at Madison in 1928 and 1929 with a view to obtaining further data bearing on the results obtained in the field experiments.

INOCULATION EXPERIMENTS IN 1928–29

Kernels from 10 selfed ears of strain A–1237, which had matured at Bloomington on plants that apparently were resistant following seed inoculation in 1928 (fig. 7), were available for these greenhouse inoculation experiments. Care was taken to obtain as uniform an inoculation as possible, a spore suspension being used. The inoculated kernels were planted in large flats in three greenhouses. One greenhouse was relatively cool (approximately 20° C.); the other two houses were warmer. An attempt to maintain the latter at a uniform temperature of 26° with a wide difference in humidity was not entirely successful. The first plantings were made in the two high-temperature houses. The second experiment utilized space in all three houses. The results are given in Table 7 and are based on 50 kernels in each case, 10 in each of the 5 groups. It is recognized that such populations are entirely too small to give data adequate for anything but indications. Nevertheless the results may serve to suggest certain differences in substrain reactions.

Of the 10 ears from supposedly resistant plants, Nos. 4 and 9 showed the largest number of healthy plants. There was some reduction in stand following inoculation, but in the case of No. 9 neither yellowing nor reduction in size of the surviving plants occurred. On the other hand, ear No. 10 had the highest percentage of germination, 96 per cent, followed by the early appearance of yellowing and a 30 per cent reduction in stand. Moreover, the plants that survived were stunted.

TABLE 7.—*Germination, stand, and length of tops of plants grown from 50 kernels of each of 10 selfed ears of yellow dent corn of strain A–1237 that had matured on apparently resistant plants, some seed uninoculated and some inoculated with Penicillium oxalicum at time of planting in greenhouse, Madison, Wis., 1928–29*

Ear No.	Kernels germinating		Plants in final stand		Mean length of tops [a]	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
	Number	*Number*	*Number*	*Number*	*Cm.*	*Cm.*
1	23	21	20	21	26.2	24.8
2	40	41	40	26	29.9	26.2
3	36	39	30	29	31.4	25.8
4	44	41	44	38	31.3	29.8
5	12	12	10	12	[b] 18.8	23.8
6	43	43	42	35	27.9	25.5
7	32	31	28	23	31.9	26.4
8	20	22	16	12	24.2	23.7
9	39	34	37	32	32.2	32.4
10	46	50	46	35	26.9	19.4
Percentage or mean	67.0	66.8	62.6	52.6	28.1	25.8
Percentage reduction		.3		16.0		8.2

[a] Measured from crown. [b] No plants in 2 of the 5 groups.

Differences following inoculation occurred in the various groups, but little evidence of a consistent resistance appeared in these seedlings, the progeny of seemingly resistant individuals. A comparison of Tables 7 and 8 shows that the percentage reduction in stand following inoculation was the same as that in a parallel inoculation experiment in which a remnant of the seed selected for the 1928 field

plantings was used. (Table 8.) Under field conditions in 1928 the stand from this seed was reduced 47.5 per cent following inoculation with *Penicillium oxalicum*. It was from plants in this plot that had not been severely injured by Penicillium that the 10 selfed ears were selected.

The fact that the 10 parent plants had been subjected to freezing temperatures in the latter part of September, 1928, was a factor not considered at the time. It has developed from the more recent field experiments (Tables 5 and 6) that plants from seed produced on mother plants injured by cold immediately prior to complete maturity are distinctly less resistant to injury from Penicillium than are those from seed produced on mother plants not so injured by cold prior to complete maturity.

TABLE 8.—*Germination and stand of yellow dent corn of strain A–1237 grown from uninoculated seed and from seed inoculated with a spore suspension of Penicillium oxalicum at time of planting in greenhouse, Madison, Wis., 1928–29*

Strain of corn	Germination		Final stand	
	Uninoculated	Inoculated	Uninoculated	Inoculated
Composite [a] A–1237, number	158	150	153	128
Per cent	79	75	76.5	64
Percentage reduction		5		16.3

[a] Remnant of seed from the 1928 field plantings.

INOCULATION EXPERIMENTS IN 1929–30

Indications of different degrees of resistance among individual plants again were evident in the field during the summer of 1929. Plants grown from ears selected from such mother plants were tested for relative resistance to *Penicillium oxalicum* in the greenhouse at Madison during the winter of 1929–30. The results are given in Table 9.

Numerous diseased plants appeared among both the controls and the inoculated plants. In the cases where the stand was reduced following inoculation the remaining plants were considerably shorter than the controls. Thinning of the stand by Penicillium injury did not result in an increased height of the surviving plants in the highly susceptible lots, although it may account for the greater height of the control plants in the more resistant selections, where the number of inoculated plants surviving exceeded the number in the controls. However, the populations represented in Tables 7, 8, and 9 are so small that the results can be regarded only as indications.

The data from the field experiments indicate that some strains are distinctly more resistant to *Penicillium oxalicum* than others. Within such strains distinct substrain differences in resistance also were found.

TABLE 9.—*Data on the reaction of young corn plants grown in the greenhouse at Madison, Wis., December, 1929, at two temperatures from seed selected from Penicillium-resistant and Penicillium-susceptible progenies of two 13-year inbred strains of yellow dent corn, some seed uninoculated and some inoculated with P. oxalicum at time of planting*

YOUNG CORN PLANTS GROWN AT 20° C.

Inbred strain No.	Progeny No.	Reaction to Penicillium, in field, of progenies, from which ears were selected	Treatment of seed	Dead	Blighting	With normal leaves	Mean height of apparently normal plants	Percentage decrease in height following inoculation
				Number	*Number*	*Number*	*Cm.*	*Per cent*
A–956 [a]	1	Very susceptible	Uninoculated [b]	2	3	10	37.5	
			Inoculated [b]	13	1	2	21.5	42.7
	2	do	Uninoculated	5	1	9	36.8	
			Inoculated	7	6	2	13.5	63.3
	3	Moderately resistant	Uninoculated	0	1	15	34.6	
			Inoculated	2	0	14	24.9	28.0
	4	do	Uninoculated	2	1	12	26.7	
			Inoculated	2	2	12	22.3	16.5
A–1237 [a]	1	Moderately susceptible	Uninoculated	1	4	11	31.9	
			Inoculated	0	2	14	30.2	5.3
	2	do	Uninoculated	0	4	9	29.8	
			Inoculated	1	2	11	27.8	6.7
	3	Highly resistant	Uninoculated	0	2	13	34.9	
			Inoculated	0	2	14	33.6	3.7
	4	do	Uninoculated	3	1	12	34.6	
			Inoculated	1	0	15	33.5	3.2

YOUNG CORN PLANTS GROWN AT 26°C.

A–956 [a]	1	Very susceptible	Uninoculated [b]	8	0	8	35.6	
			Inoculated [b]	15	0	1	20.0	43.8
	2	do	Uninoculated	9	2	5	34.8	
			Inoculated	14	1	0	0	100.0
	3	Moderately resistant	Uninoculated	1	1	15	35.3	
			Inoculated	7	2	7	31.0	12.2
	4	do	Uninoculated	0	1	6	30.5	
			Inoculated	8	2	6	26.5	13.1
A–1237 [a]	1	Moderately susceptible	Uninoculated	4	0	11	38.0	
			Inoculated	0	0	16	35.0	7.9
	2	do	Uninoculated	3	0	12	40.0	
			Inoculated	3	0	12	38.7	3.3
	3	Highly resistant	Uninoculated	5	0	10	41.8	
			Inoculated	3	0	3	37.8	9.6
	4	do	Uninoculated	7	1	7	38.6	
			Inoculated	3	0	13	38.3	.8

[a] In general, as shown in Tables 1 and 3, inbred A–1237 has been found to be more resistant to *P. oxalicum* in the field than inbred A–956.

[b] In each uninoculated and in each inoculated series, 16 kernels were planted.

HISTOLOGICAL STUDIES

METHODS

Inasmuch as the invasion of host tissue appeared limited in proportion to the damage done, it seemed advisable to determine the location of the mycelium and its effect upon host tissues.

The material examined was killed in medium chrom acetic, Juel's, Gilson's, or formol acetic alcohol fixative. Chrom acetic fixative followed by Flemming's triple stain proved most satisfactory for the host tissue. Juel's or Gilson's fixative followed by safranine, Delafield's haematoxylin, and Orange G, though not so effective for the host tissues as chrom acetic fixative and triple stain, gave a sharper contrast between host and fungus. Pianese stain following the killing

solutions already mentioned was fairly satisfactory for contrast at the time of staining. However, the colors did not prove to be permanent.

The plants studied in this phase of the problem were grown in the greenhouse in autoclaved soil or in the laboratory under sterile conditions in test tubes or Petri dishes. From plants grown in soil, adjacent or alternate sections were plated and fixed and every plant was discarded which did not give a pure culture of Penicillium. Thus it was reasonably certain that any fungus found in the remaining sections was Penicillium.

RESULTS

Location of Fungus

Tufts of the greenish fungus usually were visible at the hilum end of the kernel and along the embryo within the ruptured pericarp of seedlings which had been inoculated at time of planting by immersing the seed in a heavy suspension of Penicillium spores. As the endosperm was digested, Penicillium was often evident also upon the scutellum. (Fig. 8.) In the most severely injured seedlings, hyphae were present in the vascular cylinder near the cotyledonary plate. In a few instances they were found within the vessels. (Fig. 9, A.) *Penicillium oxalicum*, however, does not appear to show a selective entrance into the vessels as do the Fusaria causing the wilt diseases of other plants. (Fig. 10, A.)

In many specimens a mat of mycelium occupied the space between the coleorhiza and the scutellum. (Fig. 10, B.) Thence hyphae spread to the coleorhiza and eventually to the scutellum. Penetration of roots old enough for the exodermis to have assumed a protective function appeared to be difficult. In young rootlets the hyphae entered between the cells of the cap or of the cortex just back of the tip. (Fig. 11, B.) Ruptures in the cortex, produced by emerging roots, provided another avenue of entrance. (Fig. 11, A.)

In diseased plants similar to those shown in Plate 1, the fungus was found to have entered the seedling through the more or less inactive and depleted scutellum and to have advanced to the vascular tissue in the cotyledonary plate region. (Figs. 9, B, and 10, A.) In the plants that were yellowed, as shown in Plate 2, the fungus was not found in the roots, mesocotyls, or cotyledonary plate regions. It was located in the spongy tissue at the hilum end of the kernel and in the endosperm, massed in the space between the scutellum and primary root, and was entering the adjacent portions of the scutellum. (Fig. 10, B.) In all probability a fair percentage of these plants would have recovered their normal green color and developed to maturity had they been growing under favorable field conditions. It seems apparent that a certain amount of chlorosis may be produced by the absorption of the metabolic by-products emanating from a vigorous growth of the fungus even though there be little actual invasion of the young plant.

The presence of an active growth of the fungus on the seedling immediately after germination favored severe injury. When a drop of a spore suspension was placed on the embryo, soon after the rupture of the pericarp, a more severe lesion resulted than occurred when seedlings a week older were similarly inoculated.

Corn seedlings, 12 days old, grown at 28° C. A, from uninoculated seed and B, from seed inoculated with *Penicillium oxalicum* at time of planting

Corn seedlings, 12 days old, grown at 24° C. A, from uninoculated seed and B, from seed inoculated with *Penicillium oxalicum* at time of planting. The uninoculated control is at the left. The two plants at the right show symptoms of a mild infection and possibly might have regained their green color and developed to maturity.

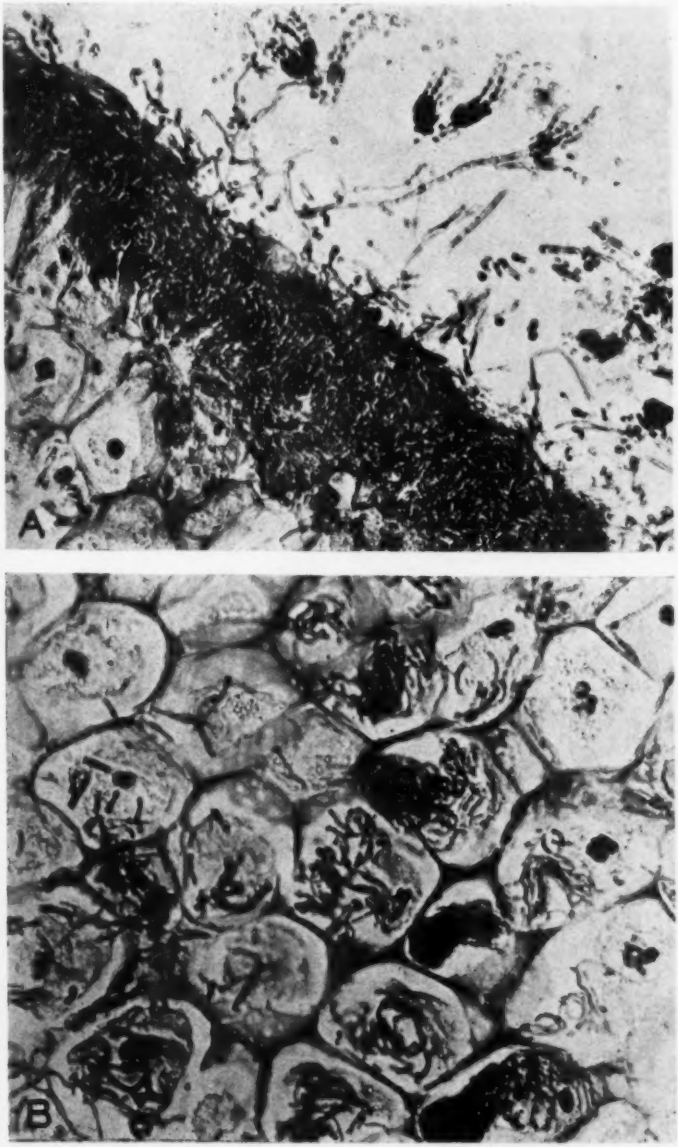

FIGURE 8.—Photomicrographs showing *Penicillium oxalicum* within the scutellum of a corn seedling grown from seed inoculated at time of planting. A, Conidiophores of *P. oxalicum* on the surface of the scutellum. The epithelial cells are indistinguishable in the mass of hyphae. × 425. B, Hyphae within plasmolyzed cells of the scutellum. (From the same section as A.) × 425

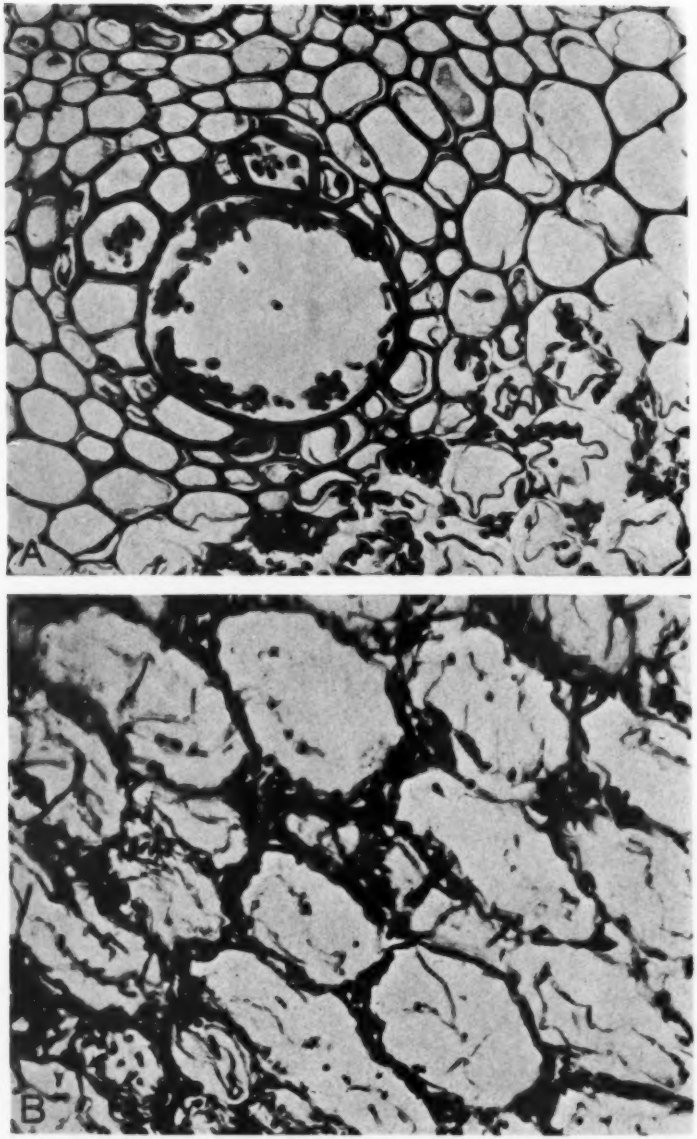

FIGURE 9.—A, Cross section of a primary root near the cotyledonary plate, showing hyphae of *Penicillium oxalicum* within vessels and plasmolyzed cells; B, intercellular hyphae in the cortex of the cotyledonary plate region adjacent to the scutellum. × 475

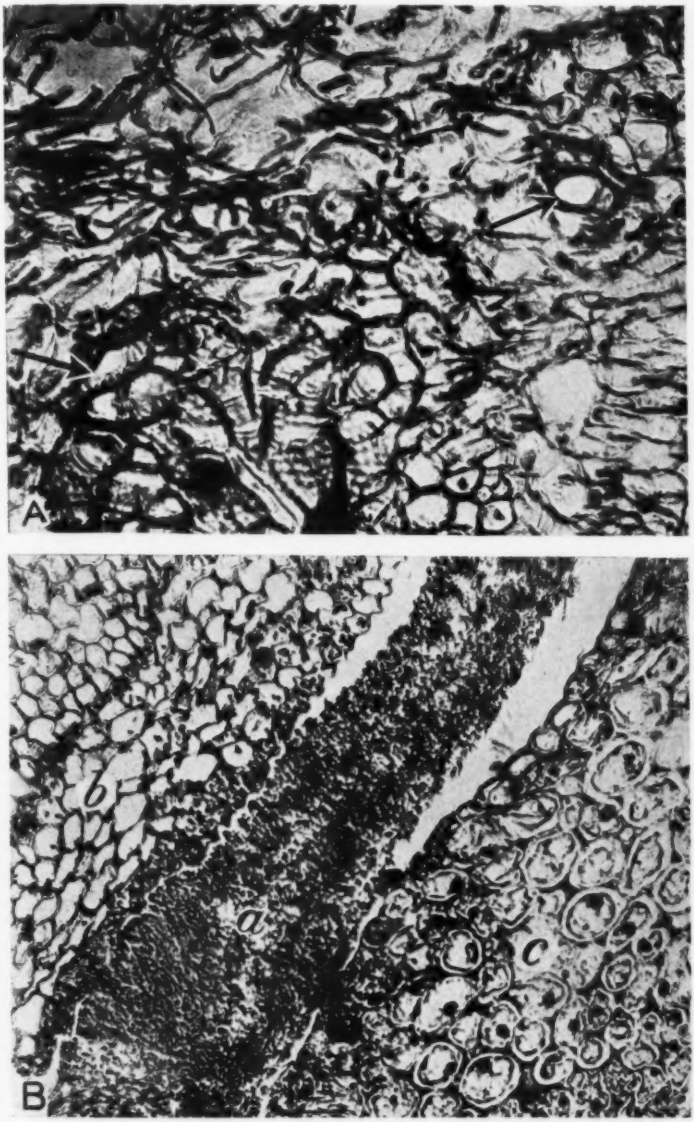

FIGURE 10.—A, Hyphae of *Penicillium oxalicum* in the cotyledonary plate region of a 15-day-old corn seedling (var. Leaming) grown at 28° C. The hyphae surround the vessels (marked by arrows) but seldom enter them. × 425. B, *P. oxalicum* (*a*) occupying the space between the coleorhiza (*b*) and the scutellum (*c*). Hyphae from this mat have penetrated both the coleorhiza and scutellum. × 200

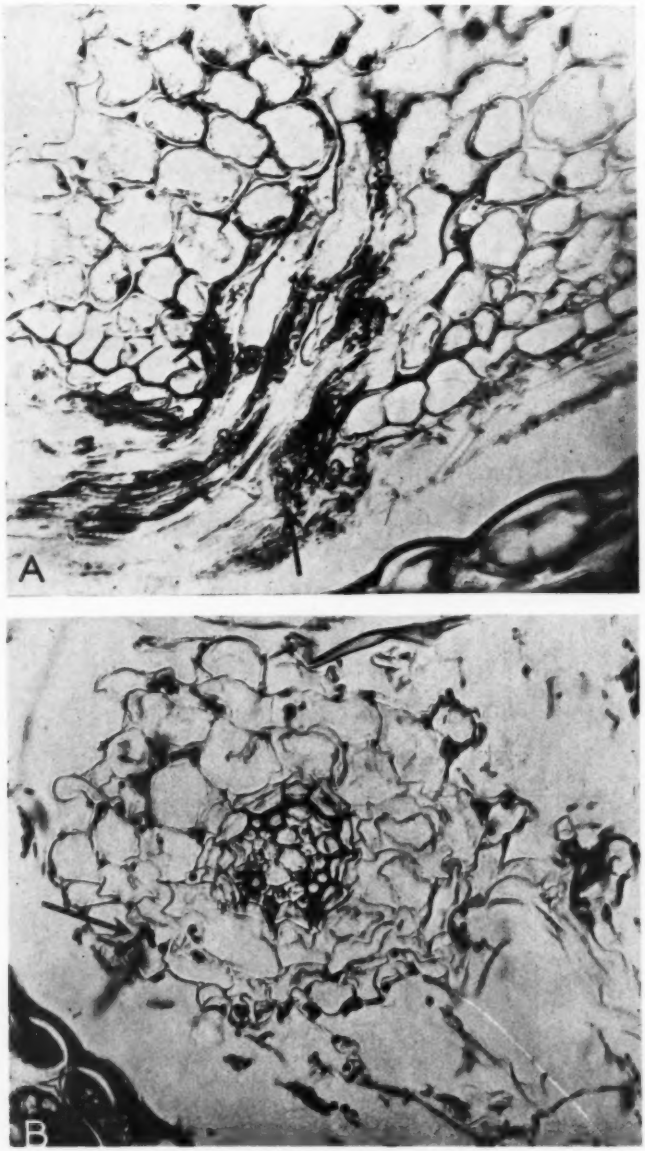

FIGURE 11.—Root penetration by *Penicillium oxalicum*: A, Entrance of hyphae through a root rupture. × 300. B, intercellular penetration of a young rootlet. × 285

In naturally infected kernels Penicillium was located for the most part in the spongy connecting tissue at the hilum end of the kernel. Slight ruptures in the pericarp at the distal end also afforded a harbor for the fungus. Germination was somewhat retarded when such kernels were plated on potato-dextrose agar. (Fig. 12.) A study of the sections of seedlings (fig. 12, *a* and *b*) showed that the fungus did not enter the young plants until after the rupture of the coleorhiza by the emerging primary root, when penetration of the tip of the primary root by the fungus occurred.

Even though the cortex of the young roots may be thoroughly permeated by the fungus, no instance of hyphal penetration of the endodermis has been observed. The endodermis seemed to be an

FIGURE 12.—Naturally infected corn kernels germinating on potato-dextrose agar after a surface treatment with zonite. Sections through *a* showed the coleorhiza to be intact; no hyphae were found within it. Penetration of the root tip of *b* occurred at points where the coleorhiza was ruptured

effective barrier to the advance of the fungus into the stele. (Fig. 13.) Hence the cotyledonary plate region is particularly susceptible to invasion due both to the absence of a distinct endodermis and to its location in respect to the storage tissues of the endosperm.

REACTION OF HOST CELLS

When lesions occur on the mesocotyl of the young plant at a time when the thin-walled cortical cells contain food material, they consist of an area of plasmolyzed cells immediately beneath the fungus. (Fig. 14, A.) Beyond this region there is a zone, one to several cells wide, in which the cell walls have become thickened, brownish in color, and have acquired a strong affinity for the safranine stain. The tex-

ture of the thickened walls is changed also. There is a tendency for them to be torn more easily by the microtome knife. Bordering these cells are others upon the walls of which are droplets of the red-staining gumlike substance. The intercellular spaces are also, at times, clogged with the same material. (Fig. 14, B.)

These droplets have a yellowish, gummy appearance before staining and may be similar to the "wound gum" of which Thatcher (*16, p. 69*) speaks when he says it

> is frequently found in the tracheæ of plants and near surface wounds, which it stanches. It is secreted by the cells surrounding the injured part * * *. These gums are generally considered to be decomposition products of celluloses, resulting from the action of some hydrolytic ferment, usually stimulated by some unfavorable condition of growth, some injury, or some morbid condition.

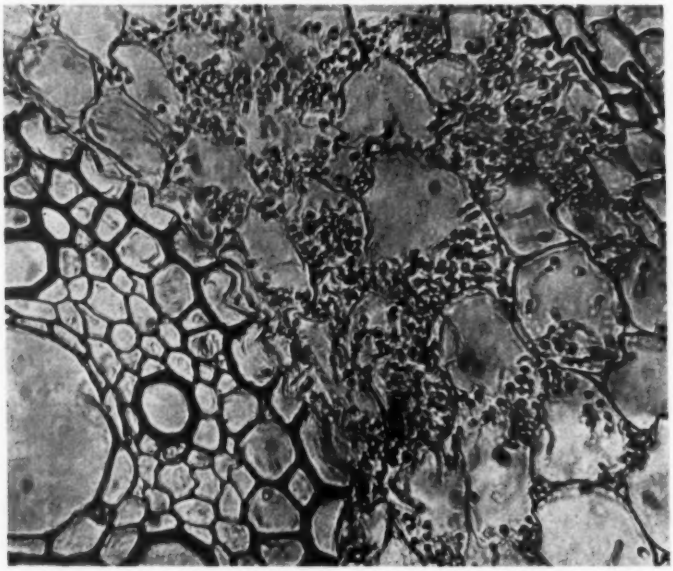

FIGURE 13.—Cross section of a young secondary rootlet of a plant grown from seed inoculated at time of planting with *Penicillium oxalicum*. Hyphae are numerous in the cortex; none, however, were observed to pass through the endodermis. Roots lying as this one did, between the mesocotyl and endosperm, are especially susceptible to invasion. × 575

Penicillium was not found to be aggressive enough to enter vigorous, living cells. Instead, it appeared to produce a substance toxic to the host cells, killing or weakening them for some distance in advance of penetration, much as described by De Bary (*2*) more than 40 years ago in connection with his studies on the parasitism of *Sclerotinia libertiana*. When a lesion is formed by a vigorous growth of Penicillium on the surface of thin-walled cortical tissue, the cells immediately beneath the fungus are plasmolyzed. Those a little farther removed from the fungus react with secretions and a change in the cell walls to the substance, which diffuses into them more slowly. Such restricted lesions when confined to the cortex do not seriously injure the seedling.

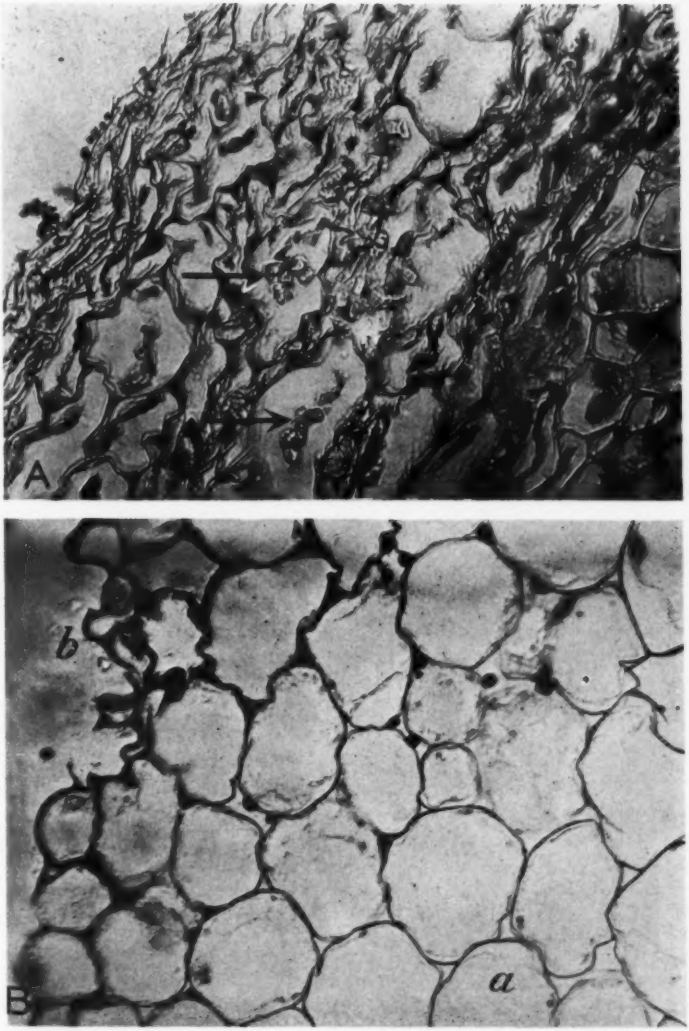

FIGURE 14.—Portions of cross sections of the mesocotyl of a corn seedling attacked by *Penicillium oxalicum:* A, Collapsed cortical cells, at the center of a lesion, with intracellular oxalate crystals (indicated by arrows). A fungus mat similar to that shown in Figure 10, B, was torn from the surface of this material during preparation for sectioning. Severe plasmolysis apparently occurred so quickly that the cell walls did not react to the toxic substance and hence did not take the safranine stain. × 425. B, Cells at the margin of a lesion produced by *P. oxalicum* showing normal condition at *a*, much shrunken condition at *b*, with walls thickened, brown when unstained, but staining with safranine, and intercellular spaces filled with gumlike substance. The cells between regions *a* and *b* are only slightly shrunken and show only slight gum formation. This gumlike substance stains blue when first formed; soon chemical changes evidently occur, after which it takes the red stain. No crystals are evident in this marginal area of the lesion. × 425

In cases of a meager growth of the fungus on the surface, plasmolysis may not occur and the cells in contact with the hyphae may react as do those in the marginal zone of the more extended lesion. If, however, conditions are such that the toxic substance reaches the vessels in sufficient quantity, yellowing of the leaves or blighting results. *Penicillium oxalicum* is known to produce a comparatively high percentage of oxalic acid from a variety of sources of carbon (*3*). This fact, together with the appearance, within 48 hours, of numerous oxalate crystals surrounding the particular culture used in these experiments when grown on potato-dextrose agar, suggested the possibility that oxalic acid might be the toxic substance responsible for the injuries observed. (Fig. 15, A.) Severe lesions were produced by placing bits of sterile cotton saturated with a 2 per cent solution of oxalic acid on the cortex in the region of the cotyledonary plate and along the base of the mesocotyl of sterile seedlings grown on water agar in test tubes. These lesions were very similar in appearance to those produced by inoculations with Penicillium. They consisted of plasmolyzed cells in immediate contact with the acid, bordered by those whose thickened walls stained deeply with safranine and still others upon whose walls appeared red-staining gummy droplets. (Fig. 15, B.)

As might be expected, when a comparatively strong solution of the acid was applied suddenly the cells in the immediate vicinity appeared to undergo a more rapid and complete plasmolysis with less wall reaction than was often the case when the lesions were produced by the presence of the fungus. The marginal cells were similar, however, in the two cases. (Figs. 14, B, and 15, B.)

It was found possible not only to produce cortical lesions by the application of oxalic acid to the surface, but also to cause retardation of growth and yellowing and drying of the leaves of young seedlings by placing them in solutions of oxalic acid ranging from 0.1 to 1 per cent. Healthy plants in the fourth-leaf or fifth-leaf stage, cut through the mesocotyl and placed in the 1 per cent solution, were killed within 24 hours. A solution of 0.1 per cent oxalic acid more nearly reproduced the effects obtained by inoculation with Penicillium. With these weaker solutions, the margins and upper halves of the leaves had become dry and brittle within 72 hours. Growth was practically stopped when the plants were placed in the solutions. The effect of the acid was more rapid and striking when the seedlings were placed in a chamber in which the relative humidity was low than when they were kept where the percentage of atmospheric moisture was high.

Experiments with *Penicillium oxalicum* by Currie and Thom (*3*), *Aspergillus niger* by Raistrick and Clark (*14*), and *Sclerotium rolfsii* by Higgins (*6*) all indicate that the composition of the nutrient medium has a direct bearing on the amount of oxalic acid produced by these fungi. Within certain limits, the amount of inoculum present is obviously also a factor in the early production of large amounts of acid. In like manner the amount of inoculum present and the nature and quantity of nutrients available to *P. oxalicum* appear to be factors in its parasitism on corn seedlings. It seems evident that, failing to produce a sufficient quantity of a toxic substance, *P. oxalicum* is unable to advance through tissues, except as a saprophyte where the cells are already dead, or along intercellular

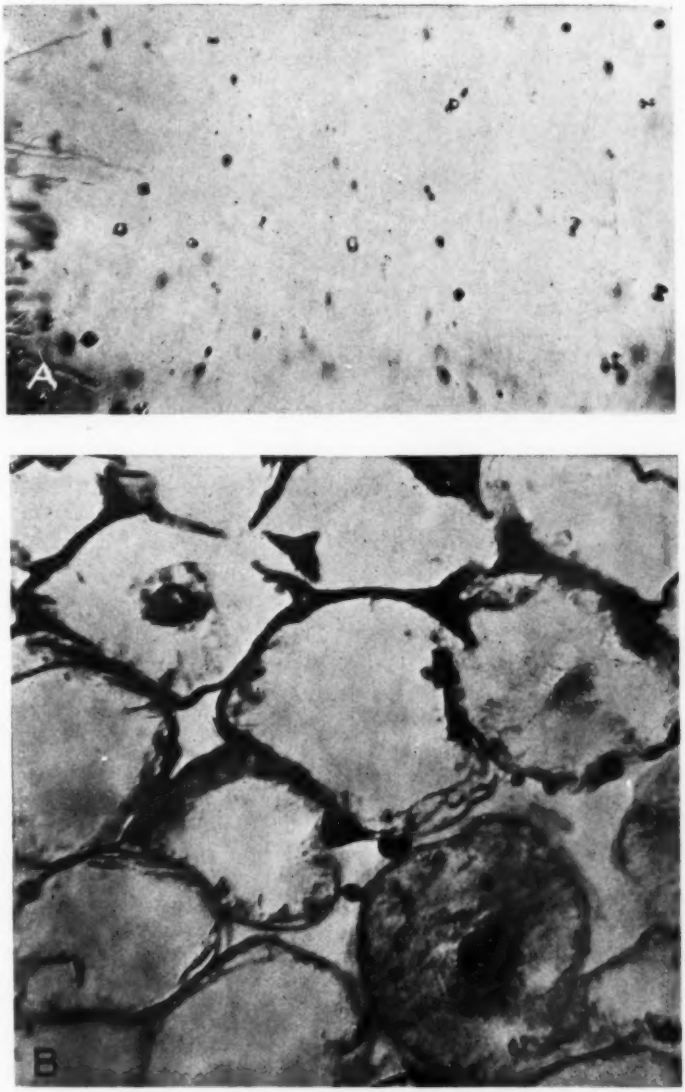

FIGURE 15.—A, Oxalate crystals in a 24-hour-old potato-dextrose agar streak culture of *Penicillium oxalicum*. The oxalic acid produced by the fungus has diffused for a considerable distance beyond the margin of the colonies. × about 175. B, Cells from the margin of a lesion produced by placing a bit of cotton saturated with a 2 per cent solution of oxalic acid on the surface of a young seedling growing on agar in a test tube. These cells have an appearance and staining reaction similar to those of cells in the same relative position in a lesion produced by *P. oxalicum*. × 525

spaces. But if given an ample supply of available carbohydrates, the increased production and subsequent diffusion of a toxic substance is an all-important aid in the parasitism of the fungus.

The reaction of the cortical cells such as described is not considered to be confined necessarily to Penicillium or oxalic acid injuries. It is rather the manifestation of a response to a certain type of injury. Much the same effect has been observed by the senior author to follow cortical invasion of the roots of corn seedlings by other fungi. Penetration by Gibberella, Cephalosporium, Basisporium, and doubtless other fungi is accompanied at times by changes in the staining reaction of the cell walls of the host and the occurrence of red-staining droplets on these walls and within the intercellular spaces. Little or no plasmolysis, however, appears to precede or accompany penetration by the above-named organisms. In fact, brownish lesions, in many cases, may be considered an indication of this type of cell reaction.

Frequent mention of gum production occurs in the literature dealing with plant diseases. Higgins (*7*) believed that gum formation is due to enzymatic activity and that it is formed in quantity only under the influence of certain abnormal stimuli. He found that treatment either with mercuric chloride or copper sulphate induced gum formation in twigs of peach, cherry, and plum.

Fawcett (*5*), though not finding it possible to reproduce all the typical symptoms of any of the gum diseases by chemical injections, induced gum formation with nitric, sulphuric, acetic, citric, and phosphoric acids, copper sulphate, mercuric chloride, and ammonium lactate, but failed with carbolic acid.

In the infected corn seedling, the gummy substance not only occurs at the margin of a cortical lesion but also often causes a more or less complete plugging of some of the vessels. The material first appears as minute droplets on the walls of the vessels and may take a deep blue color with gentian violet or Delafield's haematoxylin stain. (Fig. 16, B and C.) Later, chemical changes evidently occur, for the contents of a fully plugged vessel in most cases take the safranine stain. (Fig. 16, A.) Bartholomew (*1, p. 560–561*), when studying the formation of gum in lemon fruits, found that "the stain reactions varied all the way from different shades with pectic and cellulose stains to cases where the substance took only a lignin stain." His observations led him to suggest that the stimulus to gum formation may be a water deficiency.

STUDIES ON ACID PRODUCTION

In order to ascertain the value of the nutrients present in the young corn plants in relation to the production of oxalic acid by *Penicillium oxalicum*, decoctions were made by boiling 400 gm. of well-washed corn seedlings in the early first-leaf stage with 1,000 c. c. distilled water and filtering off the solution obtained. Seedlings from which the kernels were removed before boiling were used in making one decoction; entire seedlings, including the endosperm, furnished material for the other. To each flask containing 100 c. c. sterile solution, 1 c. c. of a decidedly green spore suspension of *P. oxalicum* was added. The cultures were incubated at 20° and 32° C. Growth was more rapid at the lower temperature, though the fungus

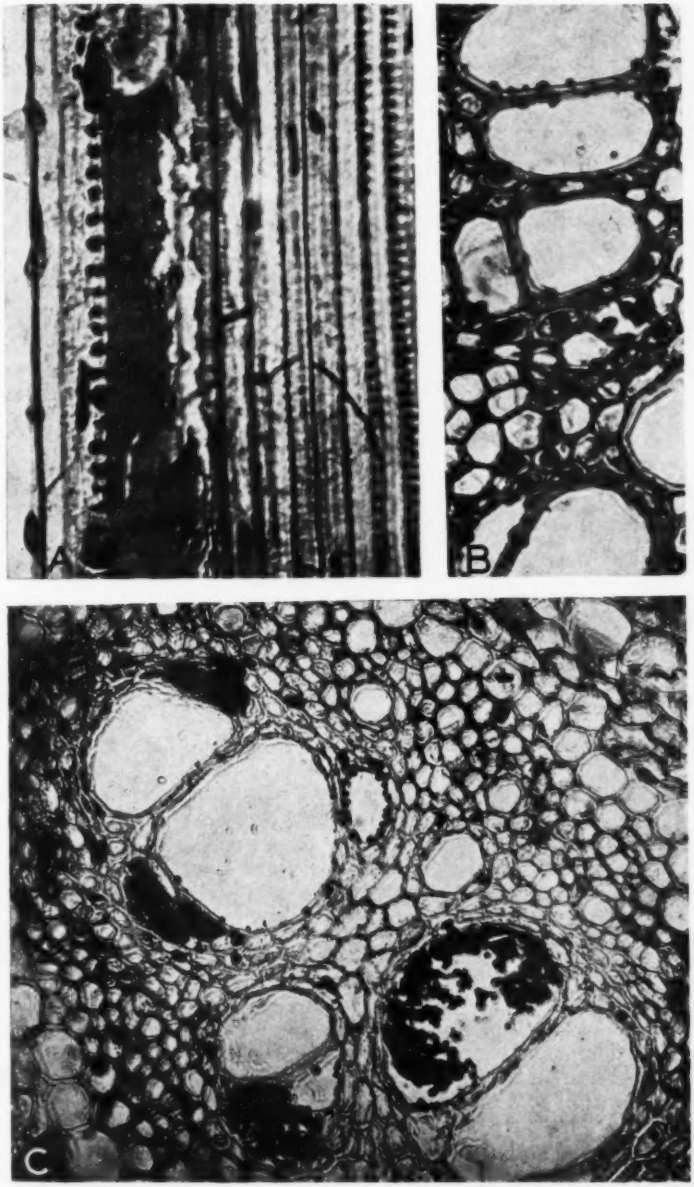

FIGURE 16.—Photomicrographs of sections of corn plants infected with *Penicillium oxalicum*, showing various stages in the plugging of the vessels. These sections were stained with safranine, Delafield's haematoxylin, and Orange G. A, Longitudinal section of a portion of a mesocotyl. The vessel and its contents stained red. × 425. B, Portion of a cross section through a mesocotyl. The droplets of gumlike substance on the walls of the vessels stained blue. × 525. C, Cross section of a rootlet. The contents of these vessels also stained blue. × 500

was vigorous at 32°. At the end of four and six days the fungus mats were filtered off on a Büchner filter and the filtrates of two 100 c. c. samples were combined for analysis.

The largest amount of oxalic acid was found in the decoction made from the entire seedlings, indicating the ability of the fungus to utilize starch in the production of oxalic acid. (Table 10.) This is in accord with the findings of Currie and Thom (*3*). In connection with their study of *Penicillium oxalicum*, these authors measured the quantity of acid produced by 23 species of the Penicillium, when grown for 14 days at 20° C. on Czapek's solution to which 5 per cent cane sugar had been added. Under the conditions of their experiment, *P. oxalicum* produced more than three times as much oxalic acid as did any of the other species. The senior author has found in this feature a helpful taxonomic character. The early appearance of numerous oxalate crystals surrounding a grayish-green Penicillium colony on potato-dextrose agar serves to suggest the presence of *P. oxalicum*.

TABLE 10.—*Oxalic acid produced by Penicillium oxalicum in decoctions made from young corn seedlings*[a]

Decoction made from—	Age	Temperature	Free oxalic acid per 200 c. c.
	Days	*° C.*	*Mgm.*
Entire seedling	4	32	66.2
Do	4	20	104.2
Do	6	20	235.2
Seedling with endosperm removed	4	20	102.5
Do	6	20	113.6

[a] The uninoculated controls in each case gave negative results. Analyses were made by Allan Dickson of the Division of Cereal Crops and Diseases and the University of Wisconsin.

EFFECT OF RATE OF TRANSPIRATION ON INJURY FROM PENICILLIUM

It has been noted that in the greenhouse the severest injury occurred at temperatures generally considered favorable for the rapid growth of corn plants, i. e., 24° to 28° C.; that, although a large amount of oxalic acid is produced at 20°, as indicated by the test solutions, comparatively few seedlings were blighted at that temperature under the usual greenhouse conditions; also, that when seedlings placed in dilute solutions of oxalic acid were allowed to remain in chambers in which the humidity was low, the effect of the acid was more rapid and more striking than when they were kept where the percentage of atmospheric moisture was high.

These observations raised the question as to whether rapid transpiration, which normally accompanies higher temperatures, might not be a significant factor in the development of the disease. If so, it would be reasonable to expect more severe injury at 20° C. under conditions favoring increased transpiration.

With this point in mind, uninoculated seed and seed inoculated with *Penicillium oxalicum* was planted in three greenhouses (Nos. 1, 2, and 3) February 9, 1929, in large flats containing autoclaved soil. The attempt to maintain two of these houses at similar high temperatures with a wide difference in atmospheric humidity was not entirely successful; hence the results presented are considered merely suggestive.

In house No. 1 the temperature was maintained at approximately 20° C. during the night except for a drop to 10° on February 18 and 19 following subzero weather outside. At midday the temperature regularly rose, a maximum of 28° being reached on March 5 and 9. Care in watering kept the relative humidity low. In this house the temperature was somewhat lower than in the other two houses, and conditions of relative humidity approached more nearly those found in house No. 2 than those in house No. 3.

In house No. 2 the temperature varied from a maximum of 38° C., which was reached for an hour on February 24, to a minimum of 25°

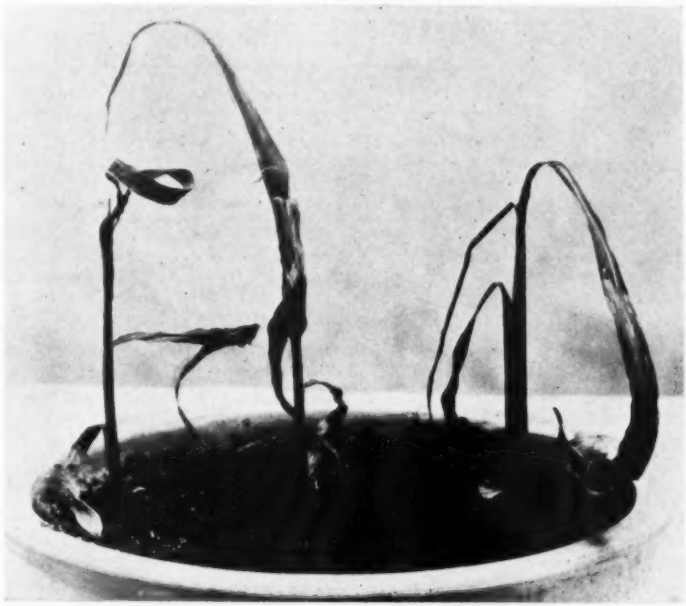

FIGURE 17.—Blighted corn plants, 30 days old, grown from seed inoculated with *Penicillium ozalicum* at time of planting. They were grown in greenhouse No. 1 (20° C., low humidity) and show typical symptoms of Penicillium injury

on February 20. The thermometer registered 30° or above near noon on 21 days. During the night temperatures usually ranged between 26° and 28°. The maximum relative humidity was 58 per cent and the minimum 30 per cent, the majority of the readings being between 40 and 50 per cent. In both houses fluctuations in temperature and humidity were greater during the latter half of the experiment because of the warm sunshine on those days.

In house No. 3 the maximum temperature recorded was 34° C. on March 7; the minimum was 23° on March 10. The thermometer rose to 30° or above on 18 of the 28 days recorded. Temperatures of 25°–27° prevailed during the nights. In this house, provided with a large-sized humidifier, the humidity ranged between a maximum of 82 per cent and a minimum of 45 per cent, with readings for the most part between 70 and 80 per cent.

The results of the inoculations with *Penicillium oxalicum* in the houses and under the conditions described are given in Table 11. They show a mean reduction in stand in houses 1, 2, and 3 of 37.2, 61.4, and 18.9 per cent, respectively, the greatest reduction occurring where the relative humidity was lowest. The plants that died in the low-temperature house (No. 1) showed the typical upright position and dried leaves characteristic of Penicillium injury. (Fig. 17.)

TABLE 11.—*Germination and stand of three lots of inbred yellow dent corn, from uninoculated seed and from seed inoculated with Penicillium oxalicum at time of planting, grown in greenhouses maintained at different temperatures and relative humidities, February 9 to March 11, 1929*

Strain	Inoculum	Germination of seed in—								
		House No. 1 [a]			House No. 2 [b]			House No. 3 [c]		
		Uninoculated	Inoculated	Percentage decrease following inoculation	Uninoculated	Inoculated	Percentage increase (+) or decrease (−) following inoculation	Uninoculated	Inoculated	Percentage decrease following inoculation
No.		*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
A–1237 [d]	Square of an agar culture.	100	100	0	97.5	100	±2.6	100	95	5
A–1237	Dense spore suspension.	97.5	95	2.6	97.5	95	−2.6	97.5	92.5	5.1
A–9	do	100	100	0	100	93.7	−3.3	100	93.3	6.7
Mean				0.9			−1.1			5.6
		Plants standing at end of experiment from seed in—								
		House No. 1 [a]			House No. 2 [b]			House No. 3 [c]		
		Uninoculated	Inoculated	Percentage decrease following inoculation	Uninoculated	Inoculated	Percentage decrease following inoculation	Uninoculated	Inoculated	Percentage decrease following inoculation
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
A–1237 [d]	Square of an agar culture.	100	65	35	97.5	37.5	61.5	97.5	75	23.1
A–1237	Dense spore suspension.	95	47.5	50	97.5	20	79.5	95	72.5	23.7
A–9	do	100	73.3	26.7	100	56.7	43.3	100	90	10
Mean				37.2			61.4			18.9

[a] Relative humidity low; temperature low.
[b] Relative humidity low; temperature high.
[c] Relative humidity high; temperature high.
[d] Forty kernels were used in all plantings of inbred A–1237, and 30 kernels were used in the plantings of inbred A–9, except in the one inoculated lot in house No. 2.

Conditions in the field at Bloomington, Ill., in the spring of 1928, also suggest that low relative humidity is more conducive to blighting than is high temperature alone. The month of May was characterized by "excessive sunshine, low humidity and rather light rainfall" (*19, v. 33, p. 17*). Plantings were made on May 9, a day on which the maximum air temperature reached 90° F. After May 10, on only five days in May did the thermometer reach 80°, 82° being the maximum. The mean temperature for the entire month was 63.2° F. (approximately 18° C.). (Fig. 3.) Despite this low mean temperature, before the end of the first week in June the plants in three plots had been practically wiped out, following inoculation with *Penicillium oxalicum*.

SUMMARY

The investigation reported in this paper was undertaken as part of a comprehensive study of the diseases of corn seedlings and deals with injury caused by *Penicillium oxalicum*.

Symptoms following inoculations in the greenhouse and field were similar, i. e., yellowing of the leaves; retardation in growth; and, in cases of severe infection, more or less complete drying of the leaves, followed by the death of the seedling. In the field at Bloomington, Ill., certain inbred strains of corn were entirely wiped out following inoculation with *Penicillium oxalicum* at time of planting. Acre yields were reduced in all cases.

Wide differences in resistance and susceptibility to Penicillium in the field were found to exist among inbred strains of dent corn, both at Bloomington, Ill., and at Madison, Wis.

In the first-generation crosses used in the field-inoculation studies in 1929, recombinations of Penicillium-resistant inbreds were highly resistant, recombinations of Penicillium-susceptible inbreds were very susceptible, and recombinations of Penicillium-resistant and Penicillium-susceptible inbreds were approximately as susceptible as the recombinations of susceptible inbreds in the series of plots on unfertilized soil.

Penicillium injuries, as measured by reductions in stands and acre yields, were greater in corn populations grown on fertilized soil than in comparable corn populations grown on unfertilized soil.

Corn grown from fully viable and nearly disease-free seed, selected from plants not injured by cold prior to maturity, was more resistant to Penicillium than corn grown from seed comparable in viability and behavior in a laboratory germination test, but selected from plants killed by cold prior to maturity.

In the greenhouse the most severe blighting occurred at temperatures of 24° to 28° C.

Penicillium oxalicum is essentially saprophytic in habit and was not found entering vigorous living cells. Sections indicate that a toxic substance that seems to be oxalic acid injures or kills the cells in advance of the fungus. The reaction of the host cells together with subsequent host invasion is described and illustrated.

The factors favoring severe infection with Penicillium appear to be those which promote an early production and absorption of comparatively large amounts of oxalic acid. Both the rate and quantity of acid formed depend chiefly on the profusion of fungal growth, which in turn depends on the amount and availability of nutrient substances. Such a food supply is available before the reserves in the corn endo-

sperm have been exhausted. If too much oxalic acid is produced before the seedling has established an independent existence, blighting is likely to result.

The suggestion is made that low relative humidity, which increases the rate of transpiration, is possibly more conducive to blighting than is high temperature alone.

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TOXIC ACTION OF AQUEOUS SODIUM CHLORATE ON NITELLA [1]

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INTRODUCTION

The experiments herein reported on the toxic action of aqueous sodium chlorate on Nitella were conducted as part of an investigative program [2] to devise economic methods for the chemical suppression of wild currant and gooseberry plants. These plants, members of the family Grossulariaceae and commonly referred to as Ribes, are the alternate hosts of the blister-rust disease of white pines, which is caused by the fungus *Cronartium ribicola* Fisch. Blister rust may be effectively controlled by hand eradication of currants and gooseberries within 900 feet of the white-pine stand delimited for protection, though in the case of certain highly susceptible species of Ribes this distance necessarily must be increased. Hand pulling is an effective eradication practice, but a cheaper method, such as chemical treatment, is needed where the plants occur in great profusion. High toxicity, low cost, and adaptability to field use are important requisites of a chemical for general use in plant-eradication work.

In field experiments more than a hundred chemicals were tested as plant poisons during the summers of 1925, 1926, and 1927 (*9*).[3] The results of these experiments showed that sodium chlorate is by far the best killing agent and in addition lends itself suitably to general field application. Sodium chlorate, fully effective on *Ribes petiolare* Dougl., is but moderately successful on other Ribes. Field experiments performed during three successive years established this difference in susceptibility very definitely and suggested that, in the mechanism of killing, specific reactions between the chlorate and the several Ribes species are involved. Practical considerations call for a chemical that will be as consistently toxic to all Ribes species encountered in blister-rust control work as sodium chlorate is to *R. petiolare*.

Thorough tests of sodium chlorate in various concentrations showed conclusively that the aqueous chlorate spray would have to be modified in some way before high toxicity to resistant Ribes could be expected. In the course of these experiments it was observed that the addition to sodium chlorate of certain chemicals, such as ammonium chloride, did not affect the toxic action characteristic of the chlorate. On the other hand, the addition to a chlorate spray of certain other chemicals, such as calcium chloride, appeared to diminish the toxic action. However, because of the inflammability of sodium chlorate when intimately mixed with organic matter, it was desirable to add to this chemical, without lowering its toxicity, a hygroscopic

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[2] Laboratory research work has been made possible through the cooperation of the College of Agriculture of the University of California, and specifically through the cooperation of the Division of Plant Nutrition and the Division of Forestry.

[3] Reference is made by number (italic) to Literature Cited, p. 809.

agent such as calcium chloride. Since variability in plant vigor, weather conditions, and other uncontrollable field factors might easily account for the observed differences between simple and modified chlorate sprays, it was recognized that an extensive program of field experiments might result in considerable loss of time unless such leads could be corroborated by laboratory work. It was decided, therefore, to investigate the toxic action of sodium chlorate under laboratory conditions; and in order to simplify laboratory technic, to insure comparable material for a series of experiments, and to reduce the number of complicating factors introduced by the plant itself, it was further decided to use some more simply organized plant than the complex and highly organized Ribes.

A review of the literature showed that previous investigations of the toxic action of sodium chlorate have been confined either to aspects of animal toxicology [4] or to field applications and greenhouse tests of the chemical as a weed killer; [5] no physiological data appear to be extant relative to the mechanism of the killing action on plants. After a survey of plant material most suitable for laboratory investigations of a physiological nature, *Nitella clavata* (Bertero) A. Br. was selected upon the suggestion of D. R. Hoagland.[6] Nitella can be conveniently collected and kept for experimental work, and it has been used with signal success by the Division of Plant Nutrition of the University of California at Berkeley, Calif., for physiological studies of the absorption and penetration of mineral constituents into plant cells.

REVIEW OF LITERATURE ON EFFECTS OF VARIOUS CHEMICALS ON NITELLA

Although Nitella and other simple plant organisms have been extensively used to study the absorption and penetration of mineral constituents into plant cells,[7] observations concerning the toxic effect of chemicals on Nitella are not so plentiful. A careful search of the literature revealed no information concerning the toxic action of chlorates on plant cells. Regarding injury of Nitella cells, however, Hoagland and Davis (*4*) noticed that an outward diffusion of chloride [8] always occurred whenever the cells were immersed in an unfavorable culture medium, and that the appearance of chloride in the immersion solution could therefore be taken as a very delicate measurement of injury. The same investigators reported (*5*) injury to Nitella cells, as shown by the appearance of chloride in the outside solution, if the cells were immersed in nontoxic solutions of calcium nitrate ($Ca(NO_3)_2.4H_2O$) and potassium nitrate (KNO_3) and a direct current of low intensity was passed through the solution. Injury to the cells was likewise apparent upon passing through a nontoxic solution a stream of air containing a high percentage of carbon dioxide (CO_2). Lowering the temperature of the immersion solution to 5° C. also caused exosmosis of chloride. Osterhout (*10*)

[4] See Sollmann (*14*) for an introduction and further references to this type of work.

[5] See reports and bulletins issued by the U. S. Department of Agriculture (1926–1930) and by many State agricultural departments in this country.

[6] Collaborator, Division of Blister Rust Control. Acknowledgment is made of the assistance and helpful suggestions given by Professor Hoagland and other members of the Division of Plant Nutrition, University of California, and of the many kindnesses rendered by A. H. Furnstal, technician.

[7] For complete bibliographies of this type of work, see the published papers of M. M. Brooks, S. C. Brooks, R. Chambers, S. F. Cook, W. J. Crozier, D. R. Hoagland and A. R. Davis, M. Irwin, R. S. Lillie, J. Loeb, W. J. V. Osterhout, and R. P. Wodehouse.

[8] Throughout this paper the term "chloride" is used by the writers to signify the chlorine present in immersion solution or in plant sap in the form of the chloride ion. This is done to avoid possible ambiguity due to the simultaneous presence of chlorate ion.

has used conductivity measurements as a means of determining injury. He found that injury of Nitella is accompanied by an increase of permeability. Regarding loss of turgidity as a criterion of injury, Osterhout (*10*) says that in isotonic or hypotonic solutions loss of turgidity indicates injury. In hypertonic solutions a similar appearance may not mean injury. In the latter case the cell recovers its turgidity when placed in tap water. Furthermore, Osterhout (*12*) states that the "time curve expressing the increase in the permeability of Nitella during the process of death is practically the same whether derived from measurements of exosmosis or of electrical resistance." In effect, therefore, the work which has just been reviewed states that injury of Nitella cells may be noted in its early stages by the outward diffusion of chloride; and that in later stages injury may be correlated with loss of turgidity, provided no recovery of the lost turgidity occurs when plasmolyzed cells are placed in tap water.

Hoagland and Davis (*4*) made some observations concerning the toxicity of ammonium salts to Nitella. They reported that the ammonium ion penetrated rapidly, the pH value of the cell sap increasing from 5.2 to 6.2 in 24 hours or less when the cells were maintained at a temperature of 25° C. At 10°, however, no exosmosis of chloride was noticed for several days. The rapidity with which ammonium salts penetrated Valonia cells and the effect of the hydrogen-ion concentration on that penetration have been fully described by M. M. Brooks (*1*). The toxicity of ammonium salts, therefore, seems to involve rapid penetration of the ammonium ion, followed by a change in the pH value of the cell sap.

The reduction in toxicity noticed in field experiments by the writers when calcium chloride was added to sodium chlorate suggested that calcium was acting in an antagonistic manner in the sense that calcium chloride is an antidote to sodium chlorate. The literature on antagonism and phenomena connected with the protective rôle which calcium plays is fully covered by Osterhout (*11*) in a recent monograph. Since it is proposed to use exosmosis as an indication of injury in this investigation, S. C. Brooks's (*2*) observation that "sodium salts increase and calcium salts decrease the rate of exosmosis of other electrolytes from the protoplasm of dandelion peduncle" is of particular interest.

A further comment concerning the effect of certain toxic chemicals on Nitella has been made by Hoagland, Hibbard, and Davis (*7*), who noticed that potassium cyanide, chloroform, and ether caused injury to the cells which resulted in decreasing their ability to concentrate substances in the vacuolar sap. The same investigators (*6*) reported that exposure of cells to iodides for 10 days or more resulted in injury which was inhibited or delayed by the presence of bromine ions. In the same paper it was noted that no accumulation of the thiocyanogen ion occurred from a nontoxic solution even over lengthy immersion periods. The toxic effect of copper on eastern Nitella was studied by Cook (*3*), who used the loss of turgidity of the cells as a criterion of the toxic action of copper chloride. The toxicity curve was found to be sigmoid, and an empirical equation was constructed which approximately fitted the curve. The toxicity was a constant fractional power of the concentration and increased with increase of temperature.

SCOPE AND METHOD OF PRESENT INVESTIGATION

The experiments with Nitella described in this paper were carried out in the laboratory of plant nutrition of the University of California in connection with the study of the toxic action of sodium chlorate on Nitella under various light conditions and in media of different pH values; the toxic action of sodium chlorate when mixed with ammonium chloride or with calcium chloride was also studied. Finally, the toxicity of sodium chlorate in the upper and lower ranges of concentration used in the preceding experiments was compared with equimolal solutions of sodium nitrate in order to evaluate the toxic action of the sodium and chlorate ions. The injury caused by ammonium chloride and by calcium chloride was determined as a check against the mixtures of these chlorides with sodium chlorate previously mentioned.

Nitella clavata was collected in October and again in April from a small lake in Golden Gate Park, San Francisco, Calif. The plant cells were placed in large wooden tubs at the point of collection and transported to Berkeley, Calif. The greater portion of the material was there placed in a cement tank and kept in tap water. The remainder of each collection was kept in large wooden washtubs filled with tap water. Under these conditions regeneration took place rapidly, and considerable cell growth was obtained even during the winter months. Healthy cells three-fourths of an inch to 2 inches long were used for the experimental work.

In all experimental work herein reported, wide-mouthed bottles (also described as packing jars), with a capacity of 4 liters each, were used as containers for the immersed Nitella. The large mouths of these jars permitted the delicate cells to be readily transferred by hand without injury. In preliminary experiments material was carefully washed in tap water and then given a final washing in distilled water. The cells were shaken vigorously to free them from all superfluous moisture and then quickly weighed and placed in the immersion solutions.[9] In all experiments it was found desirable to use as small a quantity of cells as possible in order to avoid secondary reactions caused by undue crowding of the material. Twenty-five grams of cells per liter of immersion solution was found to be a convenient quantity to use.

At the end of the immersion period, when cell sap or expressed sap was needed for analysis, the cells were removed from the solutions, thoroughly washed in distilled water, and shaken vigorously to get rid of excess moisture. Cell sap was obtained by breaking the individual cells in the open mouth of a funnel and collecting the sap in a small shell vial. This procedure is a long and tedious one, and for the most part expressed sap was used for analysis. Expressed sap was obtained by squeezing a number of cells in a small cotton bag or by crushing them in the fingers and collecting the sap as described above. With this method, some dilution of the expressed sap occurs and undoubtedly some contamination also from substances present in the cell wall and intercellular spaces; but since comparative results and

[9] In later experiments, when the effect of the hydrogen-ion concentration was being studied, it was found desirable, after washing the cells in tap water, to immerse them in distilled water overnight and to maintain the jars under the temperature and illumination conditions of the experiment before replacing with the regular immersion solution; the cells were thus allowed to come to an equilibrium under the experimental conditions.

not absolute figures were desired, the quicker technic was usually employed. Throughout the present paper, the difference between cell sap and expressed sap should be kept in mind.

The high chloride content of Nitella makes it practicable to follow by means of the outward diffusion of chloride cell injury caused by a hypotonic immersion solution, if the immersion solution contains no appreciable chloride. At regular intervals a portion of the immersion solution may be removed and the chloride determined. From an initial small value the chloride content of the immersion solution mounts more or less rapidly according to the rate of injury, and finally attains a high constant value when all the material is dead. The death rate may be determined by plotting the successive absolute amounts of chloride in parts per million against the time elapsing between the successive withdrawals of samples. In early experiments a correction was made for the change in volume caused by withdrawing a sample for analysis. In later work an equivalent volume of distilled water and buffer solution, or distilled water containing the amount of chloride shown by analysis to have been removed was added to the immersion solution. When an experiment involved immersion solutions such as ammonium chloride or calcium chloride or mixtures of these chlorides with sodium chlorate, the chloride content of the expressed sap was determined at regular intervals by removing and expressing the sap of aliquot weights of cells. At the same time, the increase or decrease in nitrogen or calcium content of another portion of the expressed sap was determined. In all cases analytical data for treated cells were compared with similar determinations made on cell sap or expressed sap of Nitella cells immersed in distilled water.

Ocular observations were made on the loss of turgidity of cells under experimental conditions; the appearance of opalescence in the immersion solutions was also noted. In addition, pH determinations of cell sap, expressed sap, and immersion solutions were made by means of the quinhydrone electrode. This electrode was not found to be entirely dependable for pH determination of cell sap and expressed sap. Frequent anomalies in readings were encountered when apparently all necessary precautions had been taken. Values for the pH of immersion solutions were quickly and accurately obtained, but are not tabulated in detail since such data do not seem of special significance.

DETERMINATION OF MINERAL CONSTITUENTS IN EXPRESSED SAP AND IN CELL SAP

DETERMINATION OF SODIUM CHLORATE

The quantitative determination of sodium chlorate ($NaClO_3$) and the effect of expressed sap and cell sap of Nitella on its recovery were first investigated. The Frankford Arsenal method (*8*) was found to give the most consistent results, and the technic of that method was used in all subsequent chlorate determinations, with the necessary changes in concentrations of reagents, as follows: One cubic centimeter of cell sap or 3 c. c. of expressed sap, diluted with distilled water to 10 c. c., and 5 c. c. 6 N sulphuric acid (H_2SO_4) are placed in a 50 c. c. Erlenmeyer flask and heated to 85° C.; 5 c. c. of 4 per cent ferrous ammonium sulphate ($FeSO_4(NH_4)_2SO_4.6H_2O$), acidified with dilute H_2SO_4, is added, and the mixture boiled for two

minutes. The liquid is cooled to 85° and titrated rapidly with standard 0.01 N potassium permanganate ($KMnO_4$).

Recovery of sodium chlorate from solutions of known concentration before and after addition of expressed sap (Table 1) was quite consistent, provided that the suspended organic matter was removed by the addition of a drop of saturated solution of alum ($KAl(SO_4)_2 \cdot 12H_2O$) followed by centrifuging. With the same treatment the addition of larger amounts of expressed sap did not interfere with the accuracy of the work, even when the amount of sodium chlorate was small. On the other hand, cell sap caused some reduction of $KMnO_4$, even after suspended organic matter had been removed.

TABLE 1.—*Recovery of sodium chlorate from solutions with and without expressed sap and cell sap of Nitella*

Solution analyzed	$NaClO_3$ added	$NaClO_3$ recovered	Error
	Mgm.	*Mgm.*	*Mgm.*
5 c. c. of distilled water	1.06	1.09	+0.03
5 c. c. of distilled water+1 c. c. of Nitella expressed sap	1.06	1.08	+.02
5 c. c. of distilled water+2 c. c. of Nitella expressed sap	1.06	1.11	+.05
5 c. c. of distilled water+5 c. c. of Nitella expressed sap	1.06	1.10	+.04
5 c. c. of distilled water+1 c. c. of Nitella cell sap	1.06	1.12	+.06
5 c. c. of distilled water+2 c. c. of Nitella cell sap	1.06	1.16	+.10

DETERMINATION OF CHLORIDE

Duplicate 25 c. c. portions of the immersion solution were evaporated to a small volume, and the chloride was determined volumetrically by Mohr's method as outlined by Scott (*13*), titrating with 0.01 N silver nitrate ($AgNO_3$). After removal of the suspended organic material by centrifuging, the chloride content of the cell sap and expressed sap was determined by the same method.

DETERMINATION OF NITROGEN

Preliminary work showed that Nesslerization, after distillation of the sap with sodium hydroxide (NaOH) or magnesium oxide (MgO), was too cumbersome and slow, and not very satisfactory because of the difficulty of obtaining ammonia-free water. The following modification was devised, which was rapid and very satisfactory. Three cubic centimeters of expressed sap were pipetted into a 15 c. c. graduated centrifuge tube. Water was added to the 14 c. c. mark, and after a short preliminary centrifuging a few drops of saturated alum solution were added. A second centrifuging gave a clear solution, which was then made up to exactly 15 c. c. and mixed by a short period of centrifuging. An aliquot part of this was Nesslerized directly by the method outlined by Scott (*13*). A set of 20 standard tubes was used for comparison, giving a range of 0.1 to 10 c. c. of standard solution (1 c. c. equivalent to 0.01 mgm. nitrogen). Fresh standards were made up every 24 hours. Generally the centrifuged sap was divided among three tubes, and each tube was compared and read. The sum of the three readings, multiplied by a factor to bring the sample to the basis of 1 c. c., gave the total amount of nitrogen in 1 c. c. of expressed sap.

DETERMINATION OF CALCIUM

For the determination of calcium a modification was used of the standard oxalate-permanganate method outlined by Scott (*13*).

The expressed sap contained much suspended and colloidal matter, such as chlorophyll and albuminous material, which proved troublesome in the calcium determination. To obviate this difficulty, the sap, after dilution with three times its volume of water, to which a trace of alum had been added, was centrifuged at top speed for 30 to 60 minutes. This procedure gave a fairly clear solution. An aliquot was drawn off, 3 c. c. being equivalent to 1 c. c. of original sap. To this was added an equal volume of saturated ammonium oxalate $(NH_4)_2$ $(C_2O_4.H_2O)$ and a drop or two of ammonia. The centrifuge tubes were placed in boiling water for half an hour and again centrifuged at top speed. By this treatment the calcium oxalate $(CaC_2O_4.H_2O)$ was thrown to the bottom of the tube. The excess $((NH_4)_2C_2O_4.H_2O)$ was decanted and the precipitate washed in centrifuge tubes three times with 10 c. c. portions of saturated $CaC_2O_4.H_2O$ solution to remove all traces of $(NH_4)_2C_2O_4.H_2O$. Each portion of $CaC_2O_4.H_2O$ wash water was centrifuged and decanted off. The washed precipitate was then dissolved in 5 to 10 c. c. of dilute H_2SO_4, transferred to 50 c. c. beakers, heated to 85° C. by placing in a larger beaker of hot water, and rapidly titrated with 0.01 N $KMnO_4$.

EARLY EXPERIMENTS ON THE TOXIC ACTION OF SODIUM CHLORATE ON NITELLA

PENETRATION INTO CYTOPLAST

Preliminary tests showed that Nitella cells partially or completely plasmolyzed by immersion in aqueous sodium chlorate did not regain their turgidity when removed from these solutions and placed in tap water. In 0.1 M solution, Nitella cells showed a definite loss of turgidity at the end of 3 hours, while loss of turgor was quite apparent after 48 hours for cells immersed in 0.01 M sodium chlorate. Nitella can be kept for protracted periods in 0.001 M sodium chlorate without appreciable injury.

Determination of the pH values of healthy cells by means of the quinhydrone electrode gave average values of 5.25 for cell sap and 5.45 for expressed sap.

After these preliminary tests were made an experiment was designed to test the rapidity with which sodium chlorate penetrated the protoplast and to note its subsequent accumulation, if any, within the cytoplast. In this experiment Nitella cells were immersed in sodium chlorate solutions having a range of concentration from hypertonic 0.1 M to hypotonic 0.00001 M. The experiment was commenced at 9 a. m. and was continued under northwest light in the laboratory. Since no artificial illumination was provided, the cells were in darkness approximately 12 hours during each 24 hours of experimentation. The immersion solutions, which were allowed to remain at room temperature, were aerated for several minutes at 4-hour intervals throughout the daylight period by a hand-operated rubber bulb fitted with an extension tube. At intervals of 48, 72, and 120 hours the amount of sodium chlorate present in the cell sap was determined according to the methods previously described. Analytical results are shown in Table 2.

TABLE 2.—*Sodium chlorate present in cell sap of Nitella after immersion in aqueous sodium chlorate under laboratory light at room temperature*

Sample No.	Molality of $NaClO_3$ solution	Period of immersion	$NaClO_3$ in 1 c. c. of cell sap	Cells completely plasmolyzed (estimate) [a]
		Hours	*Mgm.*	*Per cent*
1	0.1	48	[b] 0.07	95
2	.01	48	Trace.	20
3	.001	48	0	0
4	.0001	48	0	0
2	.01	72	Trace.	80
3	.001	72	0	0
4	.0001	72	0	0
5	.00001	72	0	0
3	.001	120	0	0
4	.0001	120	0	0
5	.00001	120	0	0
6	Distilled water (control).	120	0	0

[a] This figure does not include the cells injured mechanically in the course of the experiment. Injury was noted only in so far as it represented an increase over that of the control.

[b] Signifies amount of sodium chlorate present after allowance for reduction of permanganate caused by cell sap itself.

Definite and almost complete plasmolysis of cells in the 0.1 M sodium chlorate had occurred at the end of 48 hours, and it was only after laborious work that a small sample of the cell sap was obtained for analysis. Plasmolysis was so complete at the end of the 72-hour period that a clean sample of cell sap could not be obtained. For the same reason analysis was not made of cell sap in the case of 0.01 M sodium chlorate at the end of 120 hours. At the end of the 48-hour period, cells in the 0.01 M solution showed some injury, though the cell sap was obtained with but the usual amount of labor. In view of the healthy condition of the cells in all the more dilute solutions, and in the light of the negative results obtained upon analysis of the cell sap for the 0.001 M solution, it was considered unnecessary at the end of the 48-hour period to run a chlorate analysis upon the cells immersed in the 0.0001 M solution. As shown in Table 2, very little sodium chlorate penetrates into the cytoplast, even after considerable injury has taken place. The fact that no chlorate was found in the vacuolar sap of cells that had been immersed in noninjurious solutions for some days indicates that sodium chlorate is not accumulated, in the physiological sense, within the vacuolar sap of Nitella. Both of these facts indicate that the protoplasmic layer has a low coefficient of permeability with respect to sodium chlorate.[10] A decided browning of Nitella cells was the inevitable concomitant of serious injury caused by sodium chlorate. Cells that had been slightly injured showed an increase in the pH of cell sap from 5.2 to 5.4 shortly after injury, while the pH of the immersion solution at this time had fallen off slightly from 6.16, the original value, to 6.0.

[10] A. S. Crafts, agent, Division of Blister Rust Control, Bureau of Plant Industry, U. S. Department of Agriculture (1928–29), in the course of some physiological experiments undertaken as part of the writer's project, studied the comparative action on Tradescantia cells of isotonic solutions of: Cane sugar, (0.20 to 0.25 M); potassium nitrate, (0.12 to 0.16 M); and sodium chlorate, (0.08 to 0.12 M). Cells immersed in sugar and sodium chlorate showed little recovery. In nitrate solutions cells started to recover after 7 hours. Sodium chlorate showed injury by the outward diffusion of cell contents in 0.08 M or greater after 2½ hours. Further studies on Tradescantia with regard to isotonic coefficients, according to Lepeschin's method, showed that sodium chlorate had a low coefficient of permeability with respect to protoplasm. Sodium chlorate (0.08 M) coagulated the protoplast after 2½ hours and browned the nuclei after 30 to 36 hours. Plant cells plasmolyzed by sodium chlorate showed no recovery.

TOXIC ACTION UNDER VARIOUS LIGHT CONDITIONS

The toxic action of sodium chlorate on Nitella was observed under several light conditions by immersing plant cells in sodium chlorate solutions according to the methods previously described. The following light conditions were employed:

Laboratory light, northwest.
Red light as furnished by a specially constructed frame of red "kodaloid." (Greenhouse.)
Diffused light as furnished by a heavy white cheesecloth frame. (Greenhouse.)
Total darkness as produced by a ventilated dark box. (Greenhouse.)

The temperature of the immersion solution in the greenhouse experiments was maintained between 19° and 21° C., while that of the laboratory experiment varied between 18° and 21°. In each of the four series of experiments, the plants were in darkness about 12 hours. The injury experienced by the cells was again followed by loss of turgidity and the appearance of opalescence in the outside solution. At the end of the experiment the expressed sap was analyzed for sodium chlorate. The expressed sap rather than the cell sap was used in this experiment, since cell sap (Table 2) would not be expected to show any appreciable quantity of sodium chlorate at the end of 48 hours' immersion. Table 3 shows the quantities of sodium chlorate present in the expressed sap at the conclusion of the experiment.

TABLE 3.—*Sodium chlorate present in expressed sap of Nitella after immersion for 48 hours under various light conditions*

Molality of $NaClO_3$ solution	$NaClO_3$ in 1 c. c. of expressed sap under indicated lighting condition of—			
	Laboratory light, northwest	Red light	Diffused light	Dark box
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
0.1	1.55	1.68	0.54	0.30
0.05	.59	.72	.25	.08
0.025	.18	.26	.08	.02
0.0167	.08	.04	.02	.01

Table 3 shows definitely the presence of sodium chlorate in the expressed sap following injury of the cells, and the amounts present arrange themselves in the order of the concentrations used, being greatest in the 0.1 M and least in the 0.0167 M. Under red light and laboratory light, toxicity of sodium chlorate, as shown by the above analysis, is about the same. Diffused light provides less favorable conditions for the toxic action of the chemical. Least penetration and injury occur under dark-box conditions. The penetration of sodium chlorate into the cytoplast, even after considerable injury has occurred, is curtailed in the absence of light. The reduction in toxicity on the part of sodium chlorate at low light intensity is more striking when one considers that the cells which had been immersed in distilled water as controls showed greatest injury under dark-box conditions. Light, therefore, seems to be necessary for optimum toxicity of the chlorate.[11]

[11] In another experiment Nitella was immersed in 0.01 M sodium chlorate and 0.01 M sodium nitrate solution and irradiated for 30 minutes by ultra-violet light. In this experiment ocular observations of cells and periodic tests of the immersion solution with silver nitrate were used as criteria of injury. When the rate of injury was compared with control solutions of chlorate, nitrate, and distilled water, it was apparent that toxicity of the sodium chlorate was greatly enhanced. The increased toxicity of sodium nitrate in irradiated solution was of the same magnitude as the injury caused by the ultra-violet light itself.

Expressed sap becomes more acid, dropping from pH 5.45 to 4.6 following injury, whereas cell sap in which no chlorate was found on analysis always showed a tendency to increased alkalinity, from pH 5.25 to 5.4. A comparison of the figures in column 2 of Table 3 with those in column 4 of Table 2 shows a much larger amount of sodium chlorate in the expressed sap than was found in the cell sap. It should be borne in mind that crushing and squeezing the cells for expressed sap necessarily introduces considerable protoplast into the vacuolar sap. This method of obtaining plant sap, furthermore, is equivalent to a pressure washing of the cell wall from the inside with a slightly acid solution, the pH value of the Nitella cell sap being 5.2, and it is not unlikely that some adhered chlorate not removed by the distilled-water washing would be set free by this treatment. Cell sap, on the other hand, which is obtained by carefully rupturing individual cells, is practically uncontaminated. These data further confirm the low permeability coefficient of protoplasm with respect to sodium chlorate and suggest that the initial toxic action on the plant cell is confined to the limiting layer of protoplast.

TOXIC ACTION AS DETERMINED BY OUTWARD DIFFUSION OF CHLORIDE

The observations of previous investigators regarding the outward diffusion of chloride as a criterion of injury to Nitella cells were confirmed by the writers in a series of preliminary tests. It was apparent that the injury caused by sodium chlorate was in all cases accompanied by an exosmosis of chloride from the vacuolar sap. In order to gain an insight into the course of toxic action of sodium chlorate on Nitella with respect to time, the exosmosis of chloride was taken as a measure of the injury experienced by the Nitella cells. In this experiment the toxicities of 0.1 M, 0.05 M, 0.025 M, and 0.0167 M sodium chlorate were studied under diffused light at a temperature of approximately 21° C. The experiment was started at 8 a. m. in a greenhouse, where the immersion jars were placed under a white cheesecloth frame. In addition to chloride determinations of the immersion solutions at 6-hour intervals, the chloride content of expressed sap was determined for each series at the conclusion of the experiment. Figure 1 shows graphically the course of toxic action with respect to time.

Injury produced by sodium chlorate, as shown in Figure 1, clearly results in a rapid outward diffusion of chloride from Nitella cells. The low concentration of chloride present in the immersion solution at the beginning of the experiment includes traces of chloride from reagents as well as the chloride from cells mechanically injured in setting away the jars. Starting from this low initial concentration, the chloride curves rise rapidly to the end of the first 18-hour period. The initial action seems to be plasmolytic, and for the first 20 hours rate of injury is proportional to the concentration of the immersion solution. For the next 20 hours the toxic action follows a course which seems to be independent of the concentration of the solution. At the end of 48 hours, analysis of the expressed sap of Nitella obtained from cells that had been immersed in the 0.1 M and 0.05 M solutions showed but a trace of chloride. Similar analysis of sap from cells immersed in the 0.025 M and 0.0167 M solutions showed 80 to 230 parts per million of chloride, respectively. Expressed sap of the control showed 1,230 parts per million of chloride at the end of the 48-hour period.

TOXIC ACTION AS MODIFIED BY CHLORIDES

Ammonium Chloride

The usual quantity of Nitella cells, 75 grams to 3 liters of solution, was placed in wide-mouthed 4-liter jars which were kept under a white cheesecloth frame in a greenhouse for the period of experimentation. The diffused daylight thus obtained, and, between 5 p. m. and midnight, artificial illumination from 500-watt lamps set

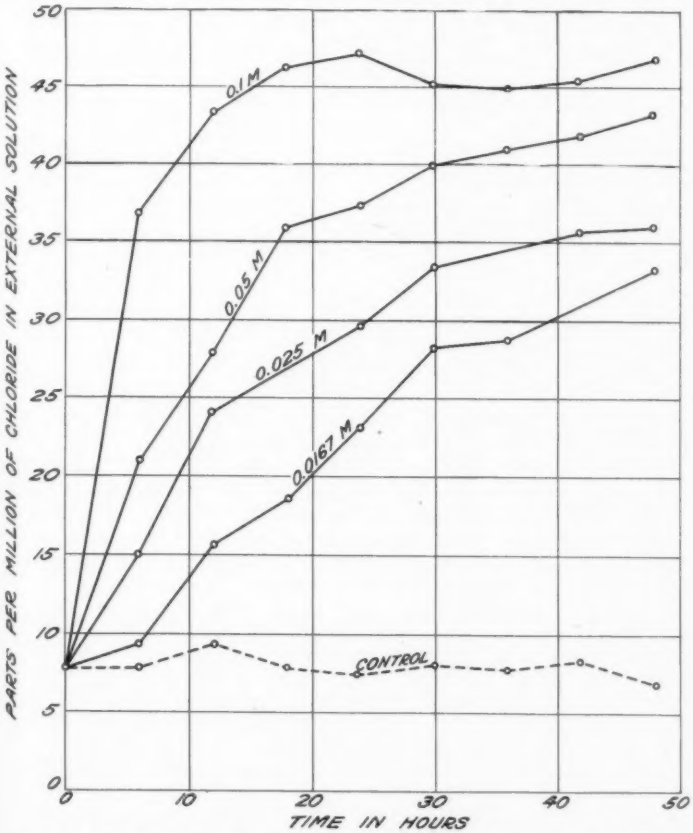

FIGURE 1.—Comparative rates of injury of Nitella when immersed in sodium chlorate solutions of different concentrations, as determined by the outward diffusion of chloride from the vacuolar sap into the immersion solution

in 16-inch Benjamin dome reflectors comprised the light conditions under which the experiment was conducted. The experiment was commenced at 8 a. m. Temperatures of immersion solutions were maintained close to 19° C. by means of a bath of running tap water.

Because of the initial high chloride content of the immersion solutions it was not practicable to follow the rate of injury, as in the preceding experiment, by periodic analysis of the immersion solution for chloride. Accordingly, observations on loss of turgidity on the

part of plant cells and the appearance of opalescence in the immersion solution were correlated with analysis of expressed sap for nitrogen, chloride, and chlorate as concrete evidence of the injury experienced by the cells. The analytical results and ocular observations given in Table 4 were recorded after 30 hours of immersion.

TABLE 4.—*Effect on Nitella of solutions of sodium chlorate, ammonium chloride, and a mixture of the two, after 30 hours of immersion, as shown by chemical analysis of the expressed sap and ocular observations*

Solution	Analysis of expressed sap			Ocular observations
	Nitrogen	Chloride	Chlorate	
	P. p. m.	*P. p. m.*	*P. p. m.*	
$NaClO_3$ (0.0167 M)	.....	1,050	29	Considerable opalescence in immersion solution and definite injury.
NH_4Cl (0.03 M)	205	800	.....	Do.
NH_4Cl (0.02 M)	240	1,020	.....	Slight opalescence in immersion solution and injury to smaller cells.
NH_4Cl (0.01 M)	175	1,480	.....	No apparent injury to large mass of cells.
NH_4Cl (0.005 M)	145	1,580	.....	Do.
$NaClO_3$ (0.0167 M)+ NH_4Cl (0.01 M).	195	850	48	Considerable opalescence in immersion solution and definite injury.
Distilled water (control)	80	1,500	.....	No apparent injury.

As noted under "Ocular observations" in Table 4, injury caused by the ammonium chloride-sodium chlorate mixture did not differ appreciably from that caused by the simple chlorate, though analytical data are indicative of somewhat greater injury in the case of the mixture. Both ocular observations and analytical data show that the addition of ammonium chloride to sodium chlorate results in a mixture which is as toxic as or even more toxic than the simple chlorate. In considering the combination of ammonium chloride (NH_4Cl) with sodium chlorate, the toxic action of ammonium chloride itself is of interest. Expressed sap from Nitella cells immersed in ammonium chloride solution showed a rapid increase in total nitrogen before injury took place. The rate of injury was proportional to the strength of the solution, being most rapid with 0.03 M and least so with 0.005 M.

It was noted that the concentration coefficients of nitrogen in expressed sap over those of the immersion solutions were in the inverse ratio of the rate of injury and strength of solution. Thus the highest value obtained for nitrogen analysis of expressed sap for each of the four ammonium chloride solutions used was: 245 p. p. m. for 0.03 M solution (concentration coefficient, 0.58) after 24 hours; 290 p. p. m. for 0.02 M solution (concentration coefficient, 1.04) after 48 hours; 390 p. p. m. for 0.01 M solution (concentration coefficient, 2.79) after 120 hours; and 255 p. p. m. for 0.005 M solution (concentration coefficient, 3.64) after 120 hours. At the end of the 120-hour period no appreciable injury had been caused by the 0.005 M ammonium chloride solution. It should be noted, by comparison, that no accumulation of chlorate ion, as such, was ever detected after immersion of Nitella cells in either hypertonic or hypotonic solutions of sodium chlorate.

Calcium Chloride

Experimental conditions and general technic were identical with those described for the previous experiment. The average analytical data for a duplicate series of this experiment and ocular observations of the cells after 72 hours of immersion are recorded in Table 5.

Table 5.—*Effect on Nitella of solutions of sodium chlorate, calcium chloride, and a mixture of the two after 72 hours of immersion, as shown by chemical analysis of the expressed sap and ocular observations*

Solution	Analysis of expressed sap			Ocular observations
	Calcium	Chloride	Chlorate	
	P. p. m.	*P. p. m.*	*P. p. m.*	
$NaClO_3$ (0.0167 M)	130	700	106	Cells completely plasmolyzed; immersion solution very opalescent.
$CaCl_2$ (0.02 M)	260	1,450	----------	Few small cells plasmolyzed; immersion solution slightly opalescent.
$CaCl_2$ (0.02 M)+$NaClO_3$ (0.0167 M).	270	1,100	46	Very little injury except to smaller cells; immersion solution slightly opalescent.
Distilled water (control)	220	1,500	----------	Slight injury to few smaller cells.

Marked plasmolysis and injury were evident in the simple chlorate solution, as usual, after 24 hours, whereas no significant injury was noticed in the case of the sodium chlorate-calcium chloride mixture until the end of the third day. Figures for chloride (Table 5) show that at the end of the 72-hour period the simple chlorate had injured the cells more than the mixture, in spite of the greater osmotic pressure of the latter. It is apparent that addition of calcium chloride ($CaCl_2$) to sodium chlorate results in an immediate decrease in toxicity. Calcium chloride here seems to be functioning in a manner antagonistic to the sodium chlorate in the sense suggested earlier in this paper (*2, 11*).

LATER EXPERIMENTS ON THE TOXIC ACTION OF SODIUM CHLORATE ON NITELLA

Influence of the Hydrogen-Ion Concentration

The toxic action of 0.01 M $NaClO_3$ on Nitella was studied at pH values of 4.8, 5.2, 5.8, 6.6, 7.4, and 8.2. The immersion solutions were made by diluting 0.2 M monopotassium phosphate (KH_2PO_4) and NaOH buffers with aerated distilled water, adding the requisite volume of molal $NaClO_3$, and finally making up to a volume of 3 liters. The resulting buffer was about 0.02 M and the $NaClO_3$ exactly 0.01 M. Recrystallized salts and chlorine-free distilled water were used in making up all solutions. After checking with the quinhydrone electrode, slight adjustments, when needed, were made with very dilute NaOH or H_2SO_4. A parallel series of controls, buffered to the same pH values, supplied a check on such toxic effects as might result from the buffers themselves.

The experiment was run in two series, in each of which three pH values were checked against their controls. Wide-mouthed jars of the usual type were immersed in a bath of running tap water by means of which the immersion solutions were maintained at a temperature between 19° and 20.5° C. during the experiment. Constant illumination was provided by a 300-watt lamp set in a 16-inch dome

reflector suspended about 18 inches above the jars. The light from the lamp was diffused through a white cheesecloth screen stretched over the jars some 6 inches below the reflector. Seventy-five grams of carefully washed Nitella was placed overnight (from 6 p. m. to 8

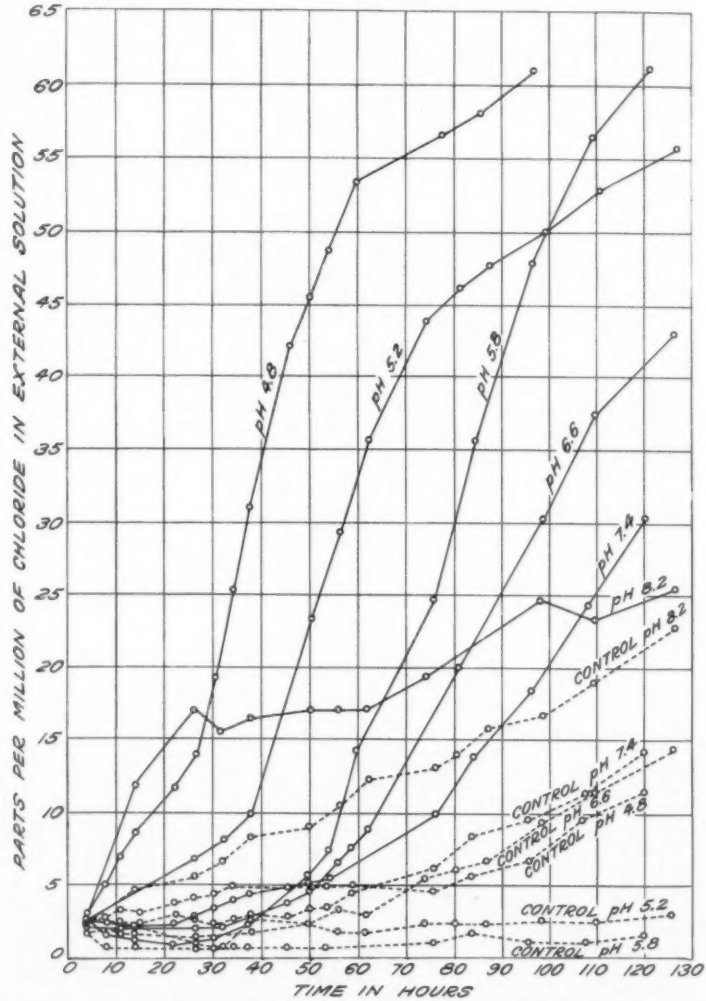

FIGURE 2.—Comparative rates of injury of Nitella when immersed in 0.01 M sodium chlorate solutions buffered to various pH values, as determined by the outward diffusion of chloride from the vacuolar sap into the immersion solution

a. m.) in each jar in distilled water, and for this period were subjected to aeration and illumination. At the beginning of the experiment the distilled water was siphoned from the jars and replaced by the experimental solutions.

The rate of injury was determined by removing small quantities of the immersion solution at convenient intervals and determining the chloride lost by the cells to the practically chloride-free medium. After each analysis a replacement was made to the immersion solutions corresponding in composition to the sample removed. The injury curves as given by the increase of chloride in the immersion solution are shown in Figure 2.

Figure 2 shows that the phosphate buffers at pH values of 5.2 and 5.8 were practically noninjurious to Nitella for the experimental period. Controls at pH values of 4.8, 6.6, and 7.4 show but slight injury. Hoagland et al. (7) previously reported that—

> Loss of chlorine accompanied by injury was not found to occur unless the pH value was below 4.8. The marked loss of chlorine and injury beginning at about pH 4.4 is correlated by Pearsall (Pearsall, W. H., and Ewing, J., New Phytologist, 1924, xxiii, 1923) with the isoelectric point of the Nitella proteins.

In a medium of pH 8.2, however, very definite injury took place. These analytical data were further substantiated by observations of the loss of cell turgidity and appearance of opalescence in the immersion solution. With the exception of the phosphate medium at a pH of 8.2, the control solutions showed little or no injury. A striking change in the toxicity of the buffer solutions was noticed when sodium chlorate was added. The values showing the time required for the outward diffusion of 50 per cent of the vacuolar chloride into the immersion solution (Table 6) illustrate quite clearly the marked difference in toxic action of acid and alkaline chlorate solutions.

TABLE 6.—*Relation of pH of 0.01 M sodium chlorate solutions to time required for the outward diffusion of 50 per cent vacuolar chloride*

Acidity of sodium chlorate solution (0.01 M)	Time required for outward diffusion of 50 per cent vacuolar chloride	Acidity of sodium chlorate solution (0.01 M)	Time required for outward diffusion of 50 per cent vacuolar chloride
pH	*Hours*	*pH*	*Hours*
4.8	38	6.6	87
5.2	56	7.4	120
5.8	81	8.2	125

It is apparent that sodium chlorate is much more injurious to Nitella in an acid than in an alkaline medium, in spite of the fact that the alkaline buffers alone are more toxic to Nitella than the acid buffers. Allowing for the slight injury caused by the buffer itself at a pH of 4.8, the rate of injury is about the same in the three most acid media. Thus, optimum toxicity is observed to occur over a pH range having an average value of 5.27, approximately the pH of the cell sap of Nitella. At the end of the 96-hour period the injury due to sodium chlorate at a pH of 8.2 was about one-third of that caused by the acid chlorate solution [12] at pH 4.8.

[12] The toxicity of acid and alkaline chlorate solutions without a phosphate buffer was studied in another experiment under light and temperature conditions similar to those in the above experiment. By continuous replacement of solution at the rate of 1,600 to 1,800 c. c. per hour, 0.01 M $NaClO_3$ immersion solutions were maintained at pH values of 4.8, 5.8, and 7.4 within ±0.2 of a pH. Since it was not practicable to regulate accurately the solution flow, the turgidity of immersed cells was noted at frequent intervals and at the same time the immersion solution was tested for chloride with dilute $AgNO_3$. After 20 hours both acid solutions had caused almost complete plasmolysis. Nitella cells had sunk to the bottom of the immersion jars. At this time cells in the alkaline solution showed only slight plasmolysis and still floated near the surface of the immersion solution. Periodic tests of the immersion solution with $AgNO_3$ further confirmed the rapid injury in acid chlorate solutions. It is of interest to record that the flowing solution of 0.01 M $NaClO_3$ caused more rapid injury to Nitella than chlorate solutions of equal strength in which no replacement of solution took place.

COMPARISON WITH SODIUM NITRATE

Solutions of sodium chlorate and sodium nitrate made from salts purified by several recrystallizations were used as immersion solutions in concentrations of 0.1 M, 0.05 M, 0.025 M, and 0.0125 M. The experimental set-up, including temperatures and illumination, was,

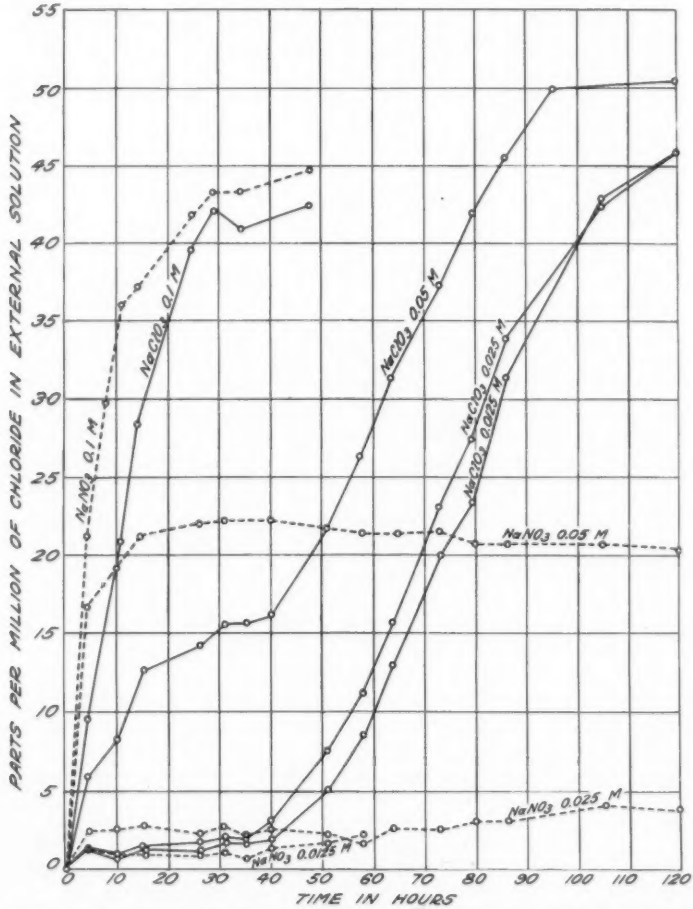

FIGURE 3.—Comparative rates of injury of Nitella when immersed in various solutions of sodium chlorate and sodium nitrate, as determined by the outward diffusion of vacuolar chloride into the immersion solution

within the limits of the methods employed, identical with that described for the preceding experiment. The rate of injury was determined, as usual, by the outward diffusion of vacuolar chloride. The average chloride content of the Nitella used in this experiment was 1,500 to 1,600 p. p. m. Young cells fell below and old cells above this average value. The analytical results are shown graphically in Figure 3.

Figure 3 shows that 0.1 M solutions of both $NaClO_3$ and $NaNO_3$ are rapidly plasmolyzing to Nitella, the action of the nitrate being even more rapid than that of the chlorate. The graph shows also that 0.05 M sodium nitrate ($NaNO_3$) is rapidly plasmolyzing to a fraction of the material, i. e., the younger cells. After this initial injury had been caused by 0.05 M $NaNO_3$, no further effect of the chemical was noted during the experimental period. Contrasted with this, the $NaClO_3$ was less rapidly injurious to the younger cells of lower osmotic value, but exerted a toxic action which increased steadily to the point of causing the death of all the material. In the 0.025 M and 0.0125 M solutions, $NaNO_3$ was not appreciably toxic within the period of 120 hours. This is in marked contrast to corresponding solutions of $NaClO_3$, which were definitely toxic after 40 hours. After 40 hours the rate of injury was the same for 0.05 M, 0.025 M, and 0.0125 M $NaClO_3$. This seems to indicate that after the initial plasmolysis of the less vigorous cells the true toxic action of the chlorate is brought into play.[13] Apparently, the typical chlorate action may be exerted over a wide range of concentrations and, after the initial plasmolytic action, the concentration does not materially affect the rate of injury.

DISCUSSION

It has been shown in this paper that not only is there no accumulation of the chlorate ion within the cytoplast when sodium chlorate acts injuriously on Nitella, but the quantity of sodium chlorate, or chlorate ion, which penetrates or is fixed by the protoplast is very small. Consequently, the original concentration of chlorate in the solution is not significantly lowered in the course of this toxic action. If this fact may be applied to a vascular plant, it follows that a sodium chlorate solution sprayed on the aerial portions of such a plant, while injuring the superficial layer of cells, is not thereby reduced to a concentration subtoxic to underlying tissue. As the superficial layers of cells become plasmolyzed and protoplasm shrinks away from the cell walls, it is probable that the chlorate solution finds easier access to the underlying tissue and that a considerable portion of the chemical may reach the vascular system of the plant. By continuation of this process, a toxic quantity of chlorate would be available for more extensive movement through the vascular system. For vascular plants a practical demonstration of the validity of this point was obtained in the course of experiments with species of Ribes. An aqueous solution of sodium chlorate 5 to 10 per cent by weight was fully effective on *R. petiolare*. Below 5 per cent a reduction of toxicity was noted; no increase of toxicity was obtained by raising the strength of chlorate solution above 10 per cent. Thus the question of concentration in relation to optimum toxicity is important, and field experiments designed to test the effectiveness of sodium chlorate on any plant should include a sufficient number of experiments to determine the trend of killing power with change of concentration.

The experiments described in this paper suggest that light intensity and wave length of light are limiting factors in the toxic action of chlorates on Nitella cells. In field experimentation, where ecological forms of a vascular plant may vary from those fully exposed to those

[13] In the course of field experiments the writers have observed that a plant fully susceptible to sodium chlorate is killed by concentrations of the chlorate (applied as an aqueous spray) ranging from 5 to 40 per cent by weight.

heavily shaded, the relationship between concentration and optimum killing efficiency should be established for maximum and minimum light conditions.

It has been shown that ammonium chloride added to sodium chlorate increases, or at least does not decrease, the toxicity of the original sodium chlorate; whereas calcium chloride very definitely functions as a protective agent. As an explanation of this reduction in toxicity by calcium chloride it has been suggested that the phenomenon of "antagonism of ions" is involved when sodium chlorate and calcium chloride are mixed together. Considerable physiological work has been done in the field of antagonism, and such work may be extremely suggestive when hygroscopic chlorate mixtures are being considered for field use. In field practice it has been considered advisable to modify the sodium chlorate by the addition of a hygroscopic agent to reduce the fire hazard.[14] On the basis of experiments reported here, it is suggested that aqueous solutions of deliquescent chlorates such as those of magnesium or calcium would be more effective than the same chlorates formed by metathesis of sodium chlorate with magnesium or calcium chloride, since the latter hygroscopic mixtures involve possible antagonism between the cations.

Sodium chlorate has been shown to be more toxic in acid than in neutral or alkaline medium.[15] Furthermore, it seems likely that the optimum toxicity of sodium chlorate solution occurs at a pH value approximately that of the cell sap. This suggestion may be readily tested by field experiments after determining the pH value of the plant sap under consideration.

The fundamental toxic action of sodium chlorate is vested in the chlorate ion, although this toxicity possibly may be modified by physicochemical relationships with the sodium ion. Therefore, chlorate compounds other than the sodium should be excellent plant poisons.[16]

SUMMARY

Sodium chlorate, or the chlorate ion, does not accumulate, in the physiological sense, within the cytoplast of Nitella.

Protoplasm has a low coefficient of permeability with respect to sodium chlorate. Penetration into the cytoplast of Nitella, even after considerable injury has taken place, is very slow. Such penetration appears to take place even more slowly when light intensity is reduced.

Injury of Nitella cells by solutions of sodium chlorate is manifested by (1) outward diffusion of chloride from vacuolar sap, (2) loss of turgidity, and (3) appearance of opalescence in the immersion solution.

[14] In the writers' experience the addition to sodium chlorate of a hygroscopic agent like calcium chloride does not insure a noninflammable mixture. The spontaneous combustion of chlorates when mixed with organic matter appears to be a very complex problem. The absorption of light energy, particularly from the ultra-violet region of the spectrum; the type of crystal formed by the drying of aqueous solutions; the temperature of decomposition of the particular chlorate under consideration; the nature of the organic material with which the chlorate is mixed; and the state of division of both chlorate crystals and particles of organic matter are factors which should be considered along with air temperature and relative humidity. The whole question of the fire hazards of the various chlorates should be given a great deal more thought and experimentation.

[15] This point has been confirmed by extensive field experiments subsequently undertaken. Conflicting data are sometimes obtained under field conditions, but in most instances Ribes that are susceptible to the lethal action of sodium chlorate are more readily killed by sprays which are slightly acid.

[16] The effects on Ribes of aqueous solutions of magnesium, sodium, iron, calcium, and barium chlorates in equimolal concentration with respect to chlorate were tested by spray application under greenhouse conditions. Although these tests were made on an insufficient number of plants to be conclusive, a decreasing order of toxicity was indicated, with magnesium and sodium first, iron second, and calcium and barium third. Apparently the toxicity of the chlorate ion is modified to some degree by the associated cation.

Ammonium chloride, or the ammonium ion, as determined by the increase of total nitrogen in expressed sap, accumulates rapidly and from a 0.005 M solution reaches a concentration coefficient of 3.6 at the end of five days. Addition of ammonium chloride to sodium chlorate results in a medium as toxic as or even more toxic than the simple sodium chlorate.

Mixtures of sodium chlorate and calcium chloride are less toxic than sodium chlorate alone. Apparently calcium chloride exhibits its well-known protective action when in combination with sodium chlorate.

Sodium chlorate is decreasingly toxic to Nitella at pH values of 4.8, 5.2, 5.8, 6.6, 7.4, and 8.2.

The fundamental toxicity of sodium chlorate may be attributed to the anion. When equimolal solutions of sodium chlorate and sodium nitrate that are not immediately plasmolytic are used, the chlorate solution is shown to be many times more toxic than sodium nitrate.

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THE NONPROTEIN NITROGEN OF THE ALASKA PEA, WITH SPECIAL REFERENCE TO THE CHEMICAL NATURE OF HUMIN NITROGEN[1]

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INTRODUCTION

The pea (*Pisum sativum*) and its seeds, in various stages of growth, have been studied by a number of investigators. Thus, Osborne and his associates (*19, 20, 21, 23*)[2] have isolated from pea seeds four proteins, namely, the globulins legumin and vicilin, the albumen legumelin, and a proteose. They have also reported the hydrolytic products of vicilin (*24*), legumelin (*25*), and legumin (*22*). From the work of Schulze and his collaborators it is known that the compounds choline (*34*) and trigonelline (*35*) occur in pea seeds. Sure and Tottingham (*37*), who studied the metabolic changes taking place in pea seeds during germination, concluded that amides accumulate in the shoot during all stages of germination, especially in the later stages when amino acids are diminishing. On the other hand, α-amino acids accumulated rapidly during the earlier stages of growth but decreased in later stages. From this they concluded that α-amino acids serve for amide production in the nitrogen metabolism of the etiolated pea plant. According to Boswell (*2*), the growth and ripening processes in pea seeds are characterized by a rapid decrease of sucrose, total soluble nitrogen, amides, basic nitrogenous substances, amino acids, and materials which produce humins upon hydrolysis; by an increase in starch, hydrolyzable polysaccharides, and insoluble nitrogen; and finally by a less rapid decrease in total nitrogen.

Humin nitrogen appears to be intimately interrelated with the more general humus question, so much so that there is some confusion in the literature regarding the occurrence and nature of the black substances. Thus, the terms "humin" and "humin nitrogen" are not infrequently employed to designate soil humus and its nitrogen as well as the humin and its nitrogen formed by hydrolysis of proteins. This is due to the fact that the humin nitrogen of the soil is derived principally from proteins and their degradation products through the action of acids or enzymes, just as the humin nitrogen discussed in the present paper is formed by hydrolysis of proteins and their products with mineral acids or through the activities of enzymes. Both types of humin are black or brown in color. In both cases, moreover, the proteins are primarily decomposed to amino dicarboxylic acids, such as aspartic and glutaminic acid; to diamino acids, such as histidine, arginine, and lysine; to monoamino acids, such as leucine, isoleucine, alanine, tyrosine, phenylalanine, tryptophane, etc. However, while

[1] Received for publication Apr. 30, 1931; issued November, 1931.
[2] Reference is made by number (italic) to Literature Cited, p. 823.

these degradation products when formed by hydrolysis of proteins remain unchanged, when formed in the soil they undergo further disintegration. For instance, the amino dicarboxylic acid, aspartic acid, in the process of putrefaction through deamination, is first converted in the soil into succinic acid, which, on splitting off carbon dioxide (decarboxylization), is further changed to propionic acid; while the amino acid, glutaminic acid, is converted into butyric acid through simultaneous deamination and decarboxylization, according to the following schemes (*1*):

$$\underset{\text{Aspartic acid}}{COOH.CH_2.CH.NH_2.COOH} \xrightarrow{(+H_2)} \underset{\text{Succinic acid}}{COOH.CH_2.CH_2.COOH}$$

$$(+NH_3) \rightarrow \underset{\text{(Propionic acid)}}{CH_3.CH_2.COOH} \; (+CO_2);$$

$$\underset{\text{Glutaminic acid}}{COOH.CH_2.CH_2.CH.NH_2.COOH} \xrightarrow{(+H_2)} \underset{\text{Butyric acid}}{CH_3.CH_2.CH_2.COOH}$$

$$(+CO_2+NH_3).$$

The monoamino acid, leucine, which was shown to occur in Michigan peat soils (*26*), is through denitrification changed in the soil to leucinic acid (*43, p. 14*) according to the equation:

$$\underset{CH_3}{\overset{CH_3}{>}}CH.CH_2.CH.NH_2.COOH + NOOH =$$

Leucine Nitrous acid

$$\underset{CH_3}{\overset{CH_3}{>}}CH.CH_2.CH.OH.COOH + N_2 + H_2O.$$

Leucinic acid

The diamino acid, lysine, also found in soils (*36*), is in the process of putrefaction through decarboxylization converted into the diamine cadaverine, according to the equation:

$$\underset{\text{Lysine}}{NH_2.CH_2.CH_2.CH_2.CH_2.CH.NH_2.COOH} =$$

$$\underset{\text{Cadaverine}}{NH._2CH._2CH._2CH_2.CH_2.CH_2.NH_2} + CO_2 (18).$$

The foregoing examples may suffice to illustrate what secondary changes the primary cleavage products of proteins may undergo in the soil. In addition to the humin substances formed in the soil or on hydrolysis of proteins with mineral acids, pigments are known that occur naturally in animal and plant tissues (hair, skin, eyes, etc.). These pigments, referred to as melanins by Schmiedeberg (*28*) and others, will not be considered here.

The purpose of this paper is to report the results of experimental work showing the nature of the humin nitrogen in the Alaska pea. These results were obtained in connection with research carried on for several years with the object of ascertaining the influence of various fertilizers on the yield and chemical composition of the pea.

REVIEW OF LITERATURE

There is a great deal of information regarding humus, the black organic matter which occurs in soils. Although the existence of Mulder's (*17*) hypothetical acids, humic, ulmic, geic, crenic, and apocrenic, has never been corroborated by succeeding investigators, much definite information has accumulated within the last 25 years concerning the chemical compounds of which humus is made up. Thus, the writer has shown that Michigan peat soils and Iowa loess soils contain considerable quantities of organic matter which on hydrolysis yields acid amides and monoamino and diamino acids (*6, 7, 8, 10, 11*), and that these compounds serve as sources of ammonia in soils (*9, 13*) since on their decomposition the liberated ammonia is largely incorporated in the soil.

The writer also predicted in 1914 (*12*) that peptones and proteoses would be found to occur in the soil. Their presence was actually demonstrated by Walters (*42*) in 1915. Schreiner, Shorey, and their associates have isolated from various soils a considerable number of nitrogenous and nonnitrogenous compounds (*16, 29, 30, 31, 32, 33, 36*) the identification of which has greatly added to the knowledge of soil humus and its nitrogen. While much literature is available on the nature of humus and especially of organic soil nitrogen, adequate information is lacking in regard to humin substances and humin nitrogen as obtained on hydrolysis of materials containing protein. Thus, in 1900, Kossel and Kutscher (*15, p. 168, 169*) wrote:

> Mulder was the first to report that humin substances are formed by the action of mineral acids on proteins. The mixture of these substances, which are chemically but little understood, is now referred to as melanoidinic acid. * * * We designate the nitrogen of the substances which remain in the barium sulfate and magnesium oxide precipitates as humin nitrogen. By this designation we do not mean to state anything.

In 1901 Kossel (*14*) stated: "Finally, on cleavage of proteins, there appear products whose relations to the aforementioned groups are not known. To these belong * * * humin substances."

It appears that up to the end of his researches (1927) Kossel never took up the question of humin nitrogen with the object of finding out its chemical nature, though in subsequent work he not infrequently had to do with humin substances. Grindley and Slater (*5*) in their quantitative determination of the amino acid content of feeding stuffs by the Van Slyke method obtained results which ranged in percentage of the total nitrogen from 3.95 per cent of humin nitrogen in blood meal to 15.79 per cent in alfalfa hay. In this connection they state that "on the average, from 8 to 10 per cent of the total nitrogen of the feeding stuffs is separated in the humin, which is an unknown mixture of secondary products * * * " (*5, p. 2768*). Roxas (*27*), who heated pure amino acids (in the presence of sugars) with water or with hydrochloric acid of different strengths in order to ascertain their rôle in humin formation, came to the conclusion that tyrosine, cystine, arginine, lysine, histidine, and tryptophane are responsible for humin formation, and that proline may be involved in that reaction under certain conditions. Gortner and Holm (*4*), who also worked with pure amino acids, have maintained that the black insoluble humin of protein hydrolysis is derived from tryptophane, while the soluble humin formed in the presence of

formaldehyde is derived from tyrosine. In order to find out the chemical nature of the humin nitrogen, the writer experimented directly with the humin substances as obtained on hydrolysis of the aqueous pea extracts. The results secured are recorded in the experimental part of this paper.

EXPERIMENTAL WORK

Peas of grade No. 4 [3] grown at the Arlington Experiment Farm, Rosslyn, Va., on a plot treated with muriate of potash at the rate of 300 pounds per acre, and peas of the same grade grown on an untreated plot, were employed in these experiments. [4] The untreated plot was naturally fairly fertile, but it received no fertilizer during the entire growing season. After drying in an electric oven at 50° C. for about two days, peas from the potash-treated plot were ground in a coffee mill and passed through a 40-mesh sieve. Six 1-liter flasks, each containing 25 gm. of the ground material, received 500 c. c. each of boiling water from which the ammonia had been removed by long boiling. The flasks were then kept on a steam bath for 30 minutes, after which their contents were centrifuged, filtration being almost impossible because of the large quantity of starch present. The solid residues in the centrifuge tubes were treated for the second time with hot water in the manner described. All the extraction liquids were concentrated on the steam bath and centrifuged again. In order to free the extract from proteins, the supernatant liquid of the centrifuge tubes (about 300 to 400 c. c.) was boiled for about five minutes, then acidified with 0.5 c. c. of glacial acetic acid previously diluted with water. The extract was then centrifuged, after which the supernatant liquid of the centrifuge tubes was treated with zinc carbonate in excess and with some lead acetate and boiled for about eight minutes. The whole was then centrifuged, the supernatant liquid of the centrifuge tubes filtered through a Büchner funnel provided with two soft filters, and this filtrate refiltered through a double-folded filter, when a clear, light-brown filtrate was obtained. This was made up with water to 500 c. c., of which two portions of 10 c. c. each were oxidized according to the Kjeldahl method to determine the quantity of nitrogen present in the extract.

An equal quantity (500 c. c.) of purified extract was similarly prepared from peas grown on the untreated plot, and the total nonprotein nitrogen was determined in the same manner as before.

NITROGEN OF ACID AMIDES

Four portions, a, b, c, and d of 120 c. c. each, of the purified extract of peas grown on the potash-treated plot, and another four portions, a′, b′, c′, and d′, from peas raised on the untreated plot, were transferred, respectively, to eight 1-liter Kjeldahl flasks. To each of these was carefully added, with constant stirring, 3.6 c. c. of concentrated sulphuric acid, making about a 5 per cent solution (by weight) of sulphuric acid. The flasks with their contents were then placed in glycerin baths and boiled under reflux condensers for two hours at

[3] Peas of No. 4 grade will not pass through a screen having round holes eleven thirty-seconds of an inch in diameter but will pass through holes twelve thirty seconds of an inch.

[4] The writer wishes to acknowledge his indebtedness to V. R. Boswell, senior horticulturist, Division of Horticultural Crops and Diseases, for supplying the pea samples used in this investigation.

about 135° C. On cooling, the contents of each flask was almost neutralized with sodium hydroxide solution and the ammonia distilled with cream of magnesia into receiving flasks containing tenth-normal sulphuric acid. The results obtained are recorded in Table 1.

TABLE 1.—*Acid amide nitrogen in the Alaska pea, expressed as percentage of the total nonprotein nitrogen in the purified extract from peas grown in potash-treated and in untreated soil*

Portion	Extract used for distillation	Total nonprotein nitrogen	Acid amide nitrogen	
	C. c.	*Mgm.*	*Mgm.*	*Per cent*
GROWN IN POTASH-TREATED SOIL				
a	120	417.528	47.2277	11.31
b	120	417.528	48.2084	11.55
c	120	417.528	47.7881	11.45
d	120	417.528	47.7181	11.43
Average			47.7356	11.43
GROWN IN UNTREATED SOIL				
a′	120	259.752	20.1604	7.76
b′	120	259.752	19.7401	7.60
c′	120	259.752	19.2077	7.39
d′	120	259.752	19.6280	7.56
Average			19.6841	7.58

NITROGEN OF AMINO ACIDS

The residues from acid amide nitrogen determinations which remained in the Kjeldahl flasks and which, in addition to magnesium oxide, contained also humin substances whose chemical nature will be demonstrated later, were repeatedly extracted with boiling ammonia-free water. The extracted materials were filtered and thoroughly washed. All filtrates and washings from the residues a and b, representing the peas from the potash-treated plot, were together concentrated on the water bath to a small volume. The resulting dark-brown sticky sirup was easily soluble in water, even at room temperature, but practically insoluble in strong alcohol. When treated with 96 per cent alcohol the alcohol gradually became permeated with a considerable quantity of a salt. About 2 to 3 volumes of 96 per cent alcohol were added, stirred well, and left standing overnight. The following morning the whole was filtered on a Büchner funnel containing two soft filters, the filtration proceeding rapidly. The salt on the filter was washed with 85 per cent alcohol and this was followed by extraction of the salt with alcohol in the Soxhlet apparatus until the siphoning alcohol appeared almost colorless. The alcohol-moist salt, which weighed 41 gm. and was completely soluble in cold water, was identified as sodium sulphate mixed with an insignificant quantity of magnesium sulphate. All filtrates, washings, and extracts were united and further concentrated on the water bath to small volume. This was filtered through a double folded filter and made up with water to 100 c. c., one-half of which (m), 50 c. c., was used directly for the determination of the amino nitrogen according to the method

of Van Slyke, and the other half (n), 50 c. c., was employed for the estimation of the peptide nitrogen.

Two portions of 10 c. c. each of m were oxidized by the Kjeldahl method to ascertain the total nitrogen present. In two other portions of 10 c. c. each the amino nitrogen was estimated by Van Slyke's method.

The extracts, filtrates, and washings from the residues a′ and b′, representing the peas from the untreated plot, were separately concentrated on a water bath and made up to 100 c. c. While the first 100 c. c., a′, was used directly for the determination of the amino nitrogen, the other 100 c. c., b′, was reserved for the estimation of peptide nitrogen. Two portions of 25 c. c. each of the solution a′ were Kjeldahlized in order to ascertain the total nitrogen present. In two other portions of 10 c. c. each the amino nitrogen was estimated by the nitrous acid method of Van Slyke. The results are presented in Table 2.

TABLE 2.—*Amino nitrogen in the Alaska pea, expressed as percentage of total nitrogen in extract of humin substances from peas grown in potash-treated and in untreated soil* [a]

GROWN IN POTASH-TREATED SOIL

Portion and analysis No.	Total nitrogen in 50 c. c. of solution	Amino nitrogen in 50 c. c. of solution	
	Mgm.	*Mgm.*	*Per cent*
m (1)	234. 18	14. 1050	6. 01
m (2)	235. 16	14. 2450	6. 07
Average	234. 67	14. 1750	6. 04

GROWN IN UNTREATED SOIL

Portion and analysis No.	Total nitrogen in 100 c. c. of solution	Amino nitrogen in 100 c. c. of solution	
	Mgm.	*Mgm.*	*Per cent*
a′ (3)	206. 00	17. 660	8. 56
a′ (4)	206. 56	17. 101	8. 29
Average	206. 28	17. 381	8. 43

[a] The figures in this table represent the average of at least two determinations.

HUMIN NITROGEN FORMED ON HYDROLYSIS OF POLYPEPTIDES

According to Fischer (*3*), the hydrolysis of polypeptides proceeds in a manner quite similar to that of proteins, as far as strength of acids and time necessary for hydrolysis are concerned. Accordingly the 50 c. c. of solution n, representing the potash-treated plot, and two 50 c. c. portions of solution b′[5], representing the untreated plot, all of which were reserved for the peptide nitrogen estimations, were transferred to 800 c. c. Kjeldahl flasks.[6] To these enough concentrated hydrochloric acid was added to make a concentration of 20 per cent, and the whole boiled in glycerin baths at a temperature

[5] The 100 c. c. of solution b′ was divided into two 50 c. c. portions, m′ and n′.

[6] Large flasks are necessary because at the beginning of hydrolysis the substance foams badly.

of about 130° C. for 12 hours, reflux condensers being used. Each hydrolysate which contained a considerable quantity of black substance was then diluted somewhat with water and filtered separately through a Büchner funnel containing one hardened filter. The humin substance was washed thoroughly with hot, ammonia-free water. Oxidized by the Kjeldahl method the humins of portions n and n′ gave 50.38 and 12.0906 mgm., corresponding respectively to 21.47 and 11.72 per cent of the total nitrogen present as humin nitrogen.

NITROGEN OF POLYPEPTIDES

The filtrates and washings from the humin substances were evaporated in the water bath to complete dryness in order to get rid of the hydrochloric acid. The dark-brown residues were moistened with a few drops of acetic acid, taken up with hot water, filtered, and made up to 50 c. c. In two 10 c. c. portions of this solution the total nitrogen was estimated according to the Kjeldahl method. In two other 10 c. c. portions the amino nitrogen was determined by Van Slyke's nitrous acid method. The data secured are reported in Table 3.

TABLE 3.—*Peptide nitrogen in the Alaska pea, expressed as perceniage of total nitrogen in extract of humin substances from peas grown in potash-treated and in untreated soil* [a]

GROWN IN POTASH-TREATED SOIL

Portion and analysis No.	Total nitrogen in 10 c. c. of solution	Peptide nitrogen plus amino nitrogen (originally present in solution) in 10 c. c.		Peptide nitrogen [b]
	Mgm.	*Mgm.*	*Per cent*	*Per cent*
n (1)	32.657	14.340	43.91	37.87
n (2)	33.400	14.512	43.45	37.41
Average	33.029	14.426	43.68	37.64

GROWN IN UNTREATED SOIL

Portion and analysis No.	Total nitrogen in 50 c. c. of solution	Peptide nitrogen plus amino nitrogen (originally present in solution) in 50 c. c.		Peptide nitrogen [b]
	Mgm.	*Mgm.*	*Per cent*	*Per cent*
m′ (3)	81.6085	40.6610	49.59	41.16
m′ (4)	82.3790	41.2180	50.27	41.84
Average	81.9938	40.9395	49.93	41.50
n′ (5)	80.5575	40.689	50.58	42.15
n′ (6)	80.3475			
Average	80.4525	40.689	50.58	42.15
Average of m′ and n′			50.26	41.83

[a] The figures in this table represent the average of duplicate determinations.
[b] On the basis of the nonprotein nitrogen.

NITROGEN OF DIAMINO AND OF MONOAMINO ACIDS

The residues c and d, which remained in the Kjeldahl flasks after the ammonia had been distilled off (see p. 815) were thoroughly

extracted with boiling ammonia-free water and freed from humin by filtration. The filtrates on concentration were separated from the sulphates of sodium and magnesium as already described and the final concentrated solutions made up with water to 100 c. c. In two portions of each solution of 10 c. c. each the nitrogen was estimated by the Kjeldahl method. The remaining 80 c. c. portions were made up with water to 100 c. c. each. To each of these were added 5 gm. of sulphuric acid and 30 c. c. of a solution containing 20 gm. of phosphotungstic acid and 5 gm. of sulphuric acid per 100 c. c. After 24 hours the phosphotungstates were filtered out and washed in the usual manner. Portion c was used for the qualitative tests. On being freed from phosphotungstic acid it showed the following reactions:

Acidified with sulphuric or hydrochloric acid, it gave with phosphotungstic acid a heavy, grayish-white precipitate; with phosphomolybdic acid, a yellow precipitate; with mercuric chloride, in the presence of barium hydroxide, a gray flocculent precipitate; with silver nitrate, a grayish-white precipitate soluble in excess of ammonia. These reactions are characteristic of diamino acids.

Portion d was Kjeldahlized to ascertain the quantity of basic nitrogen present. Unfortunately this estimation was lost.

The filtrate from the phosphotungstate of both flasks was diluted with three volumes of ammonia-free water, almost neutralized with calcium hydroxide and then made distinctly alkaline with barium hydroxide. Next the whole was saturated with carbon dioxide, brought to a boil, then filtered on a Büchner funnel containing two soft filters and washed well with hot ammonia-free water. The residue on the Büchner funnel was again extracted with hot water. All filtrates and washings were concentrated on the water bath to small volume and filtered. The filtrate from an insoluble grayish-white residue, which was identified as a mixture of gypsum and calcium carbonate, was made up to 100 c. c. In three 10 c. c. portions of this solution the total nitrogen was determined by the Kjeldahl method, while in three other 10 c. c. portions the amino nitrogen was estimated according to the Van Slyke method. The analyses showed 4.07, 4.19, and 3.95 per cent, averaging 4.07 per cent, of monoamino nitrogen.

CHEMICAL NATURE OF HUMIN NITROGEN FORMED ON HYDROLYSIS OF ACID AMIDES

It seems best to present this subject in three parts, dealing with (1) the proportion of humin nitrogen found in the peas, (2) the cleavage of the humin nitrogen into a soluble and an insoluble fraction, and (3) the percentage of amino nitrogen found in the soluble fraction.

Proportion of Humin Nitrogen in Peas

Each of the residues a and b,[7], as well as the residues a′, b′, c′, and d′, remaining in the Kjeldahl flasks after the ammonia had been driven off (p. 865), received 25 c. c. of hot ammonia-free water, and was then brought to a boil and allowed to settle, after which the supernatant liquid was decanted on a Büchner funnel containing a hardened filter.

[7] The residues c and d were used for estimation of the diamino and monoamino nitrogen.

The treatment was repeated with each of the solid residues until the last washings ceased to give a precipitate with barium chloride. Each residue was now transferred to a Büchner funnel, washed with hot water, and the suction allowed to continue until the substance became dry. It was then mechanically removed from the filter to the Kjeldahl flask as completely as possible, traces being washed down by means of the wash bottle. The last traces were dissolved in dilute sulphuric acid and added to the same flask. The nitrogen determinations by the Kjeldahl method of the residues a and b yielded 27.0113 and 29.1548 mgm. of nitrogen, corresponding, respectively, to 6.47 and 6.98 per cent of the total nonprotein nitrogen in the form of humin nitrogen, the average being 6.73 per cent for the peas grown on the potash-treated plot.

The corresponding figures for the residues a′ and c′, from peas raised on the untreated plot, were 28.5384 and 26.0586 mgm. of nitrogen, corresponding to 10.98 and 10.03, the average being 10.51 per cent of the total nonprotein nitrogen in the form of humin nitrogen.

Cleavage of Humin Nitrogen into a Soluble and an Insoluble Fraction

The residues b′ and d′ were hydrolyzed in 1-liter Kjeldahl flasks with 100 and 200 c. c. of 37 per cent hydrochloric acid, respectively, for 12 hours. The hydrolyzing mixtures were kept boiling quietly at about 135°–140° C. At the expiration of this time the hydrolysates were evaporated on the water bath to dryness, the residues moistened with a few drops of acetic acid, then taken up with hot ammonia-free water, filtered, and washed with hot ammonia-free water. The almost coal-black humin residues which remained on the filters were transferred quantitatively to the respective Kjeldahl flasks in which hydrolysis had taken place and oxidized by the Kjeldahl method. They yielded, respectively, 11.8525 and 10.7177 mgm. of nitrogen, corresponding to 4.56 and 4.13 per cent of the total nonprotein nitrogen in the form of residual humin nitrogen. Since the total humin nitrogen obtained was equal to 10.98 and 10.03 per cent, it is evident that 41.54 and 41.18 per cent of the residual humin nitrogen, respectively, was not affected by the hydrolysis with 37 per cent hydrochloric acid, remaining as humin nitrogen under the conditions described.

Amino Nitrogen in Soluble Fraction

The filtrate and washings from the residual humins were concentrated separately on the water bath and made up to 50 c. c. After a preliminary examination of the solutions had been made, two aliquots of 10 c. c. each were oxidized according to the Kjeldahl method, and in two other portions of 10 c. c. each the amino nitrogen was estimated according to Van Slyke's nitrous acid method. The results secured were as follows: On an average, 50 c. c. of the solutions corresponding to the humin residues b′ and d′ were found to be equal to 16.6350 and 16.6020 mgm. of nitrogen corresponding to 6.40 and 6.39 per cent of the total nonprotein nitrogen in the form of soluble nitrogen, as obtained by hydrolysis with 37 per cent hydrochloric acid. The estimations, according to the Van Slyke method, gave the following results: Two 10 c. c. portions of b′ and two 10 c. c. aliquots of d′ gave, on an average, 52.36 and 52.41 per cent, respectively, of amino nitrogen. The data on the humin nitrogen are presented in Table 4.

TABLE 4.—*Humin nitrogen of the Alaska pea as affected by hydrolysis with 37 per cent hydrochloric acid*

Humin residue	Total humin nitrogen (on hydrolysis of acid amides with 5 per cent sulphuric acid)		Residual humin nitrogen (on hydrolysis of the humin substances with 37 per cent hydrochloric acid)			Soluble nitrogen (on hydrolysis of the humin substances with 37 per cent hydrochloric acid)			Residual humin nitrogen plus soluble nitrogen			Amino nitrogen		
	Weight	On the basis of the nonprotein nitrogen	Weight	On the basis of the nonprotein nitrogen	On the basis of the total humin nitrogen	Weight	On the basis of the nonprotein nitrogen	On the basis of the total humin nitrogen	Weight	On the basis of the nonprotein nitrogen	On the basis of the total humin nitrogen	Weight	On the basis of the soluble nitrogen [a]	On the basis of the total humin nitrogen
	Mgm.	*P. ct.* [b]	*Mgm.*	*P. ct.*	*P. ct.*	*Mgm.*	*P. ct.*	*P. ct.*	*Mgm.*	*P. ct.*	*P. ct.*	*Mgm.*	*P. ct.*	*P. ct.*
b′	28.5384	10.98	11.8525	4.56	41.54	16.6350	6.40	58.29	28.4875	10.96	99.83	8.7010	52.36	30.52
d′	26.0586	10.03	10.7177	4.13	41.18	16.6020	6.39	63.71	27.3197	10.52	104.89	8.7093	52.41	33.39
Average		10.51		4.35	41.36		6.40	61.00		10.74	102.36		52.39	31.96

[a] This soluble nitrogen was obtained on hydrolysis of the total humin nitrogen with 37 per cent hydrochloric acid for 12 hours.

[b] The figures in columns 2 and 3 refer to humin residues a′ and c′, which are, however, equivalent to humin residues b′ and d′.

It will be seen from Table 4 that there was found for b′ 4.56 per cent of residual humin nitrogen and 6.4 per cent of soluble nitrogen, totaling 10.96 per cent of nitrogen, which accords well with the 10.98 per cent of total humin nitrogen found. The figures for d′ were 4.13 per cent of residual humin nitrogen and 6.39 per cent of soluble nitrogen, totaling 10.52 per cent nitrogen, which is also in reasonable agreement with the 10.03 per cent of total humin nitrogen found. It will be noticed further that the amino nitrogen found (column 14) on the basis of the soluble nitrogen, averaged 52.39 per cent. The question naturally arises: What is the nature of the remaining 47.61 per cent? While a complete answer to this question can be given only by further investigation, it seems fairly safe to assume that a considerable part, if not all, of the 47.61 per cent is made up of amino acids also. In this connection the following facts must be borne in mind.

Roxas (*27*) has shown that tyrosine, cystine, and tryptophane, as well as the diamino acids are responsible for humin formation and that proline may also be involved in this reaction under certain conditions. These compounds, however, do not react with all of their groups on nitrous acid to evolve elementary nitrogen. Thus, Van Slyke has demonstrated (*38, 39, 40, 41*) that tryptophane reacts with one-half of its nitrogen, histidine with one-third, arginine with one-fourth, while proline and oxyproline react with none. It may be added that lysine practically reacts only with the α-amino group on nitrous acid within five minutes, the time which is ordinarily used in the Van Slyke method, while the Ω-amino group requires one-half hour for complete reaction. Consequently, the elementary nitrogen evolved by the Van Slyke method respresents only a part of the amino acid nitrogen. Whether all of the amino acids under consideration occur in the aqueous extract of the Alaska

pea has not yet been investigated. It is certain, however, that diamino acids are present, as already shown in this investigation. Furthermore, from the work of Osborne and his associates (*19*, *20*, *21*, *23*), it is known that the pea seed contains the proteins legumin, vicilin, and legumelin, all of which contain proline, tyrosine, and tryptophane, in addition to the diamino acids (*22*, *24*, *25*). These facts render it quite probable that the aqueous extract of the Alaska pea contains these amino acids. This will be readily comprehended when it is borne in mind that for the protein synthesis going on in leguminous and, generally speaking, in higher plants, not all of the amino acids are used up quantitatively. A small portion of the amino acids ordinarily remains as such in the seed either because the anabolic process has not been fully completed or because slight catabolism of the proteins takes place under the influence of proteolytic enzymes.

The percentage of amino nitrogen, calculated on the basis of the total humin nitrogen, is on an average but 31.96 per cent (last column). Undoubtedly this is due to the causes just outlined and also to the fact that the percentage of soluble nitrogen is only 61.00 per cent (column 9). It is possible that the proportion of soluble nitrogen would have been greater had methods been used different from the one employed in this work.

To facilitate a comparison of all the results obtained they are presented in summary form in Table 5.

TABLE 5.—*Nonprotein-nitrogen content of peas grown in potash-treated and in untreated soil*

	Peas grown in potash-treated soil	Peas grown in untreated soil
	Per cent [a]	*Per cent* [a]
Acid amide nitrogen	11.43	7.58
Humin nitrogen formed on hydrolysis of acid amides with 5 per cent sulphuric acid	6.73	10.51
Amino nitrogen	6.04	8.43
Humin nitrogen formed on hydrolysis of polypeptides with 20 per cent hydrochloric acid	21.47	11.72
Peptide nitrogen	37.64	41.83
Residual nitrogen	16.69	19.93
Total	100.00	100.00
Diamino nitrogen	[b]	[c]
Monoamino nitrogen	4.07	[c]

[a] Average per cent of total nonprotein nitrogen. [b] Lost. [c] Not determined.

DISCUSSION

Table 5 shows that of the nonprotein nitrogen, the percentage of acid amide nitrogen is higher in the peas grown in potash-fertilized soil (11.43) than in those grown in untreated soil (7.58), while the percentage of amino and peptide nitrogen is somewhat higher in the latter (8.43 and 41.83, respectively) than in the former (6.04 and 37.64, respectively). Of these compounds the amino acids are known to be nutritious, whereas opinions differ as to nutritive value of acid amides. The writer does not know of any experiments dealing directly with the value of polypeptides from the standpoint of nutri-

tion. However, judging from the fact that in the nitrogen metabolism of the higher plants the polypeptides stand between the amino acids on the one hand and the proteins on the other, and that both of these have high nutritive value, it seems fairly safe to assume that the polypeptides have nutritious qualities. Now the sum of amide, amino, and peptide nitrogen differs very little in the peas from the two plots, being 55.11 per cent in peas from potash-treated soil and 57.84 per cent in those from untreated soil. For the reasons given it may be said that so far as the nutritive value of their nonprotein compounds is concerned, the peas from the two plots are essentially equal. These compounds, however, constitute but a fraction of the total nitrogen.

The percentage of polypeptides in the Alaska pea is strikingly high, as it is in some other vegetables examined by the writer. It would seem, therefore, to be very desirable that the polypeptides receive further study. The percentage of humin nitrogen, too, is considerable; a fact which suggests the importance of research with a view to finding out more about the nature of humins and especially of humin nitrogen.

From the work of Roxas (*27*) as well as of Gortner and Holm (*4*) it is known that humin is formed from certain amino acids heated with mineral acids in the presence of sugars, aldehydes, or ketones. Since the aqueous extract of the Alaska pea contains appreciable quantities of amino acids and sugar, it is easy to understand why conditions are favorable for the formation of humin. Gortner and Holm (*4*), who experimented with pure amino acids and came to the conclusion that tyrosine and tryptophane are responsible for the soluble and insoluble humin, respectively, did not deal with the chemical nature of the humin substances. Roxas (*27*), who also experimented with pure amino acids, claimed to have demonstrated that in the case of cystine, tyrosine, arginine, and lysine the humin nitrogen is due to a reaction rather than to an adsorption. He suggested that the amino acids tryptophane, arginine, and histidine have labile hydrogen atoms that enable them to give condensation products with carbohydrates, with the formation of a ring. On the other hand, Grindley and Slater (*5*) believe that with the exception of tryptophane, which enters into chemical combination with carbohydrates, the amino acids are to a greater or less extent adsorbed by the humin substances formed by the action of mineral acids upon carbohydrates. The fact demonstrated in this paper, that the humin nitrogen can be split by simple hydrolysis into amino nitrogen to the extent of about 32 per cent, renders it highly probable that the humin substances obtained from the Alaska pea have peptide linkings.

SUMMARY

Seeds of the Alaska pea (grade No. 4) grown in potash-treated and in untreated soil are shown to contain, respectively, as percentages of the total nonprotein nitrogen: Acid amide nitrogen, 11.43 and 7.58 per cent; humin nitrogen formed on hydrolysis of the acid amides, 6.73 and 10.51 per cent; amino nitrogen, 6.04 and 8.43 per cent; peptide nitrogen, 37.64 and 41.83 per cent; and humin nitrogen formed on hydrolysis of the polypeptides, 21.47 and 11.72 per cent.

Since the sum of the more or less nutritive constituents, namely, the amide, amino, and peptide nitrogen, in peas grown in potash-treated and untreated soil, is 55.11 and 57.84 per cent, respectively, it is concluded that there is no essential difference in the nutritive value of such peas so far as the nonprotein nitrogen compounds are concerned. These, however, make up but a fraction of the total nitrogen.

The humin nitrogen resulting from hydrolysis of the acid amides was shown to be split by hydrolysis with 37 per cent hydrochloric acid into a soluble portion containing 61 per cent of the total humin nitrogen in soluble form, and an insoluble portion, with 41.36 per cent residual humin nitrogen. The soluble portion contained 52.39 per cent amino nitrogen, which means that 31.96 per cent of the total humin nitrogen was converted into amino nitrogen by the hydrolysis. It is believed, however, that the actual percentage of amino nitrogen is considerably higher, since by Van Slyke's nitrous acid method only a part of the nitrogen of certain amino acids (tryptophane, proline, oxyproline, and hexone bases) is split off in elementary form. Some of these amino acids (Kossel's so-called hexone bases) have been shown actually to occur in the aqueous extracts of the Alaska pea. On theoretical grounds, as outlined in this paper, it is probable that the other amino acids occur in the extracts. Inasmuch as the humins obtained from pea materials yielded amino nitrogen on simple hydrolysis, it seems safe to conclude that they had peptide linkings.

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OLEORESIN PRODUCTION FROM LONGLEAF PINE DEFOLIATED BY FIRE[1]

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INTRODUCTION

Little information is available concerning the direct effects of forest fires on the yields of oleoresin (*1*, *2*)[3] and none, so far as is known, on the effects of these fires on the cell structure and responses inside the trees. This paper gives specific information on a test conducted to determine the effect of fire on the oleoresin yields and growth responses from young longleaf pine (*3*, *4*, *5*). The studies on the internal structure and responses of these trees were made by the Forest Products Laboratory.

DESCRIPTION OF THE TEST

A stand of longleaf pine (*Pinus palustris* Miller) about 40 years of age, a part of a tract covering over 10,000 acres of land near Pregnall, S. C., was selected for the test. The trees on the test area averaged about 200 per acre. The soil was somewhat better than that generally found in the turpentine woods of the coastal plain. A light ground fire, which consumed the surface growth consisting chiefly of broom sedge but did not reach the crowns of the trees except as noted, had run through the stand in November, 1926, just before the stand was turpentined for the first time. The information available indicates that the area had been previously burned by ground fires about once a year, as is the local custom. On an acre or more, however, the fire of 1926 had swept through the tops of the trees. As a result the foliage on a number of these trees was severely scorched and killed. The new leaves for that year, however, were still inclosed in the bud coverings and survived.

A group of 10 trees, averaging 9 inches in diameter 4.5 feet above the ground and about 50 feet in height, was selected from the defoliated trees. These defoliated trees, which throughout this paper are designated as scorched trees, were matched with a second group of 20 similar trees growing close by, the foliage of which had not been burned. In this paper, the trees in the second group are designated as unscorched trees, although fire had run through the ground cover beneath them.

Although it was recognized that the number of trees in the test was small, yet this in itself was of some advantage since the trees could be matched tree by tree, and the proximity of the two groups and the uniformity of the site on which they grew eliminated variations from this cause.

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[2] The author wishes to make acknowledgment to Austin Cary, of the Forest Service, who planned the test and selected the trees used. Further acknowledgment is made to the Southern Railway Co., owner of the timber, and especially to J. F. Wynn, its forester, who weighed the oleoresin and assisted in collecting the specimens studied.
[3] Reference is made by number (italic) to Literature Cited, p. 836.

The external appearance of the scorched and unscorched trees in April, 1927, is shown in Figures 1 and 2. The scorched trees had by that time developed a sparse foliage from the buds that escaped the searing effect of the November fire.

The methods of chipping were those used in ordinary commercial turpentining and were applied by whatever chippers were chipping through the crop in which the test was located. Both groups were chipped by the same chipper at a given chipping. The chipping, which was approximately one-half to three-fourths inch deep and one-half inch high, was not of the most conservative type with reference to height, depth, and width of face, which was about as wide as the

FIGURE 1.—External appearance of the scorched trees five months after the fire. The new leaves were just pushing out from the buds that survived scorching

diameter of the tree, but only one face was placed on each tree. The same methods were applied to both the scorched and the unscorched trees. During the test, some of the scorched trees died and other like trees were substituted for them so that there were always 10 scorched trees from which oleoresin was obtained. No identifying numbers were given individual trees in each group. The oleoresin from all the trees in a group was weighed together at each dipping or collection.

After the 1926 fire, the entire stand was successfully protected from fire during the period discussed here, which ended at the close of the turpentining season in December, 1929. The fire hazard of

the abundant undergrowth, as it appeared at the end of the summer of 1927 following the November burning of the tract, is shown in Figure 3. By 1929, however, the character of the undergrowth on the tract had changed so that all that remained by November of that year was a flattened mat of dead vegetation and pine needles, which served excellently to hold moisture and to promote good tree growth (*6*). At this time the undergrowth no longer caused appreciable obstruction to progress through the woods.

Samples of wood were removed from the scorched and unscorched trees and studied microscopically for the purpose of observing the effect of fire and of turpentining on the cell structure of the wood

FIGURE 2.—External appearance of the unscorched trees five months after the fire

and inner bark. Some of them were in the form of chips cut with a hack in the regular process of turpentining from midway between the peak and the shoulder of the streak at the top of the face. Others were in the form of blocks cut out with a knife and chisel. The samples were about 1 inch wide and as deep as the streak, that is, 0.5 to 0.75 inch. They showed the wood formation at the midstreak position of the current chipping, a position which has been found to present a fair average of the conditions existing along the streak (*7*, *8*). The specimens were cut progressively higher and higher on the tree as the chipping advanced upward. The chips collected on a given date were always cut from approximately the same height on all the trees.

EFFECTS OF SCORCHING FOLIAGE ON TREE VITALITY AND GROWTH

GROSS INJURIES RESULTING FROM THE FIRE

During the first year of the test three of the badly scorched trees died. Some scorched trees even when unturpentined also died. Two of the scorched trees in the test died in May when only 10 streaks had been cut. The third did not succumb until July, at which time it had been chipped seventeen times. Another tree in this group, although it remained alive, ceased to exude oleoresin after about 17 streaks had been cut. By the end of the 1927 season, in December, still another tree had dry-faced, although its top was still green.

FIGURE 3.—High undergrowth produced the summer following the fire

When the second season, that of 1928, started, four of the original scorched trees were dead, and one was alive but dry-faced so that it yielded no oleoresin. All the unscorched trees were alive and exuding oleoresin. It appears therefore that at the beginning of the second season 50 per cent of the faces on the original scorched and turpentined trees had had their oleoresin-producing power destroyed.

During the third season, 1929, one more scorched tree began to show dry-face. By the end of that year only one-half of the face was yielding oleoresin so that the total number of productive faces remaining from the 10 original faces on the scorced trees was only four and one-half.

The foliage of the surviving scorched trees, however, in the autumn of 1929 appeared as vigorous and well developed as that of the unscorched trees (*5*).

EFFECTS OF SCORCHING ON INTERNAL STRUCTURE

The wood collected from the scorched trees on April 19, 1927, showed retarded and enfeebled formation of resin tissue, wood cells, and phloem. The 1927 growth ring immediately above the face averaged less than one row of spring wood cells, the number of rows of cells varying from a maximum of five in one tree to a minimum of no wood formation whatever in other trees. In only one tree was the beginning of resin-tissue formation for the year apparent. The phloem from the scorched trees also appeared abnormal. In contrast, the unscorched trees averaged four rows of spring wood cells with a maximum of seven or eight; in one-third of the trees, developing resin passages were also present. Even by August 12, 1927, the

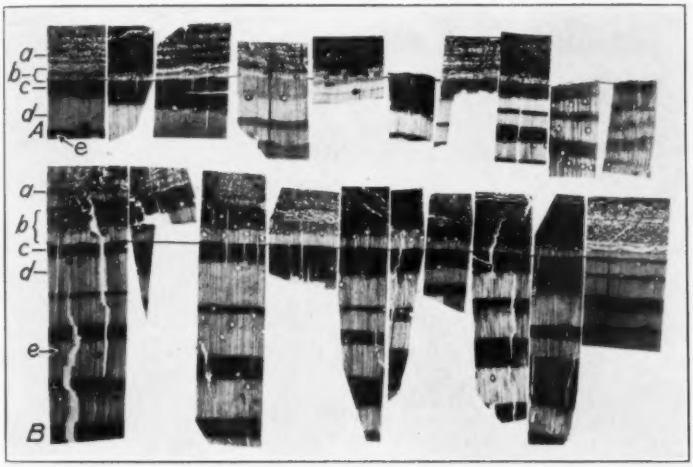

FIGURE 4.—Cross sections of chips from midstreak at the top of the face at the end of the first year of turpentining. All sections are arranged with the widest ringed specimens at the left and the narrowest at the right to show the range in variation among the trees in the contrasted groups. The beginning of the 1927 ring in each specimen is used as the horizontal base line, the year's growth during turpentining being shown in varying widths above it. A, Chips from trees which had foliage scorched and killed November, 1926. Of the 5 trees represented by the sections farthest to the right, 3 were dead and 2 unproductive. B, Chips from adjacent unscorched normal trees which show the usual responses to turpentining, such as a somewhat narrower growth ring and increased numbers of resin passages. All tree swere alive and productive. *a*, Inner bark or phloem; *b*, 1927 annual-growth ring; *c*, summer wood; *d*, spring wood typical of wood formed before turpentining began; *e*, resin passage

wood formation for that year was still markedly retarded in the scorched trees.

The examination of the specimens cut November 22, 1927, at the end of the first year of turpentining further demonstrated the injury resulting mainly from the fire. Cross sections of the wood from these specimens as they appeared on a horizontal plane extending about one-eighth to one-half inch into the tree above the face are shown in Figure 4. The white streaks are cracks caused by the shattering blows of the hack with which the wood was removed. The reduction in wood formation in the 1927 annual-growth ring in the scorched trees as compared with the unscorched trees is clearly illustrated. The poorest unscorched tree made about as good growth as the best of the scorched trees.

Figure 5 illustrates the wood formation in the scorched and unscorched trees on November 26, 1929. Figure 5, A, shows sections from representative trees of those that survived the scorching. They include specimens from both the original scorched trees and from the additional scorched trees substituted for those that died or ceased to produce. All have markedly less wood formation and resin-yielding tissue than the normal unscorched trees shown in Figure 5, B. The combined width of the three seasons' growth of wood in the chips removed from the unscorched trees averaged several times the width of that in the scorched trees. The varying degrees of development and recovery express the results of the efforts made by these handicapped trees to live and function.

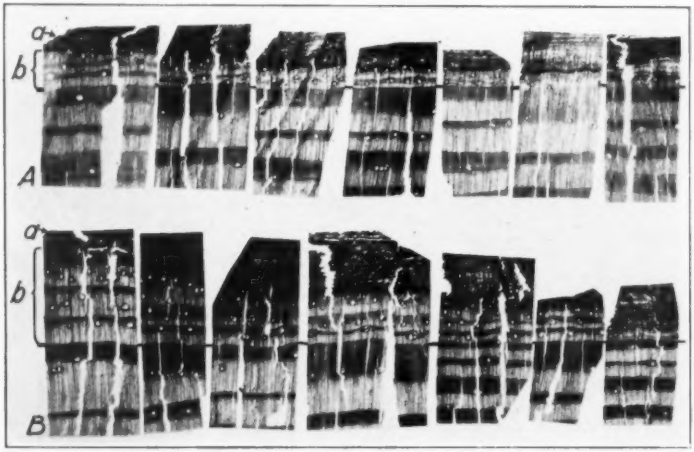

FIGURE 5.—Cross sections of chips from midstreak at the top of the face at the end of the third year of turpentining. All sections are lined up as in Figure 4 with the beginning of the 1927 growth ring as a base line. A, Chips from scorched trees. Note that the portion indicated by the brackets has less than three annual rings in most of the specimens as well as reduced ring width and reduced summer-wood and resin-tissue development, and many of the phloem cells are empty. There is an approach to normal conditions in the 1929 ring next the bark. B, Chips from unscorched trees. Note the three annual growth rings indicated by the brackets which show the usual responses to turpentining and also the phloem stocked with reserves. *a*, Inner bark or phloem; *b*, the 1927 to 1929 growth rings formed during turpentining

The failure of the scorched trees to make normal growth is clearly evident when the reduced number and narrowness of the growth rings as well as the poorly developed summer wood of the specimens shown in Figure 5, A, are compared with the three normal rings formed by the unscorched trees. In 6 specimens out of 10 from the living scorched trees only two complete growth rings were formed, and in 2 others barely one ring was formed in the wood above the turpentined face during the 3-year period. The reduction in wood formation was also apparent opposite the faces on the scorched trees where the total width of the three growth rings (1927–1929) was more than 40 per cent below the width of the rings of wood formed by the unscorched trees as determined on borings made at breast height (4.5 feet above the ground). Yet here, too, the power of the longleaf pines to rehabilitate themselves is apparent when the better developed and wider ring of wood which the survivors were able to

form in the last year of the test, 1929, is compared with the earlier wood formation during turpentining.

From the data presented it is possible partly to visualize the physiological responses of the living scorched trees. With the aid of the stored reserves in their wood and phloem cells, these trees responded to the natural urge toward normal growth. Though stripped of their foliage, they put forth new crowns of leaves from the buds that had resisted the searing effects of the fire. The activities of these thin canopies of needles, in conjunction with the energy from the stored reserves, enabled them more or less successfully to develop wood and other cells. That about one-half of the original group of damaged trees was able to yield oleoresin after the fire during the first year of turpentining (1927) and at the same time to produce foliage and continue to form even a partial quota of wood cells, is evidence of the amazing power of this species to recuperate.

The specimens from the unscorched trees showed in the wood above the turpentined face the typical responses of young longleaf pine to the methods of exploitation now practiced in ordinary commercial turpentining. These include the production of a greater than normal amount of resin-yielding tissue, which generally occurs at the expense of wood formation. The growth rings formed after turpentining tend to have less summer wood, the walls of which may be comparatively thin. Had the chipping been somewhat less deep and high (*8*, *9*) (the face height was 18 inches at the end of the first year), and had the faces been somewhat narrower, better wood formation would undoubtedly have occurred in both groups.

YIELDS OF OLEORESIN FROM SCORCHED AND UNSCORCHED TREES

The weights of oleoresin obtained for the scorched and unscorched trees as dip and scrape for each of the three seasons are presented in Table 1. The more than 50 per cent reduction in the yield from the original scorched trees (p. 834), as compared with the yield of the normal trees, shows the damage and depreciation in the potential value of a turpentined stand that may at any time result from a fire.

In order to determine what would have been the yield of 10 scorched trees had all survived the fire, scorched trees from the same area as those first selected were substituted in the test as soon as the original trees died or ceased to exude oleoresin. The actual total yield of oleoresin thus obtained from 10 scorched but producing trees during three seasons, the weight of oleoresin actually obtained, is given in Table 1. A calculated yield, based on the average production per face per tree per year, is also given in order to represent the oleoresin production of the original 10 trees. Only four and one-half faces of the original 10 were actually productive by the end of 1929. The yields of the trees which died or ceased to exude oleoresin were estimated on the basis of the average yield per streak per tree of the whole group for the number of weeks during which they were producing oleoresin.

The recovery of the scorched trees with respect to oleoresin-yielding capacity is significant. The trees responded to the exploitation for oleoresin with a yield that after the first year almost equaled, tree for tree, the yield of the unscorched trees. The yield of the scorched

trees in 1929 exceeded their yield in 1927, although such was not the case with the unscorched trees.

TABLE 1.—*Yields of oleoresin from scorched and unscorched longleaf pine at Pregnall, S. C.*

Type and number of trees	Year [a]	Weight of—			Average weight of dip and scrape per tree	Calculated yield of turpentine per crop [d] (10,000 cups)	Comparison of production from scorched with that from unscorched trees
		Dip [b]	Scrape [c]	Dip and scrape			
		Pounds	*Pounds*	*Pounds*	*Pounds*	*Barrels*	*Per cent*
Scorched, 10	1927	31.00	9.00	40.00	4.0	17.9	60.7
Do	1928	21.50	14.00	35.50	3.6	14.5	96.7
Do	1929	37.00	13.00	50.00	5.0	22.0	95.2
Calculated values for original 10 scorched trees [e]	1927	22.20	6.71	28.91	2.9	12.9	43.7
Do	1928	10.75	7.00	17.75	1.8	7.2	48.0
Do	1929	18.10	6.30	24.40	2.4	10.7	46.3
Unscorched, 20	1927	[f] 104.00	26.00	130.00	6.5	29.5	100.0
Do	1928	44.00	30.00	74.00	3.7	[g] 15.0	100.0
Do	1929	78.00	27.00	105.00	5.2	23.1	100.0

[a] The number of streaks cut were 33 in 1927; 26 in 1928; and 32 in 1929.

[b] Dip is the semiliquid portion of the exudate which collects in the cups and is periodically "dipped" out.

[c] Scrape is the hardened portion of the exudate from which some of the turpentine has evaporated, it clings to the face and is scraped off at the end of each season.

[d] About 1 gallon of turpentine on the average is obtained, respectively, from 40 pounds of dip and from 75 pounds of scrape. For every 50-gallon barrel of turpentine about 3½ barrels (500 pounds gross or 420 pounds net) of rosin are produced.

[e] This is based on the average yield per weekly streak per tree of 10 scorched trees at a given collection of gum multiplied by the number of streaks which were productive on each of the original 10 scorched trees. It therefore tends to give a slightly higher yield than if individual weights of the oleoresin from each dying or dry-faced tree had been available.

[f] This seemingly high yield as compared with 1928 and 1929 is probably due to the fact that more streaks were cut in 1927, that the faces were slightly wider in the butt region, that in 1928 the cups were not raised, and that by 1929 when cups were raised these small trees had begun to feel the drain of the heavy commercial chipping used.

[g] This low yield is explained by the small size of the timber (9-inch d.b.h.) and the relatively few streaks cut and the fact that the cups were not raised. The average production in 1928 on the whole tract (16 crops) of different ages of working and sizes of trees was 35 barrels per crop.

The oleoresin yielded during the turpentining of both the scorched and the unscorched trees exuded from the resiniferous tissues exposed on the entire surface of the streak, an area of wood half an inch or more in width. The exudation therefore was not confined to the region of the wood formed during the three years when the trees were turpentined but came also from a varying number of rings which had been produced by the trees when they were unturpentined (round timber). (Fig. 5.) Such rings may contain from 25 to 100 or more resin passages per inch. Because of the narrowness of the annual rings formed during 1927, 1928, and 1929 in the scorched trees, these had a greater proportion of the streak surface made up of the normal annual rings equipped with normal resin passages than was the case in the unscorched trees. Therefore the normal resiniferous tissues were probably stimulated so that they undoubtedly played an important part in enabling the scorched trees to produce what, under the circumstances, were notably high yields. (Table 1.)

Although, as a rule, well-developed annual rings and numerous resin passages are associated with high yields, exceptions to this general condition may occur, especially when trees undergo drastic injury. Thus a tendency for severely turpentined trees to produce for a time large amounts of oleoresin at the expense of wood formation

has been frequently observed. It has also been noted in a comparison of high-yielding trees that large exudations of oleoresin may be obtained from tissues containing very poorly developed resin passages. By contrast in very exceptional cases the resiniferous tissue, although present, may yet produce little or no oleoresin. It is therefore evident that as yet no satisfactory gage, unsupported by yield data, is available of the potential oleoresin producing power of resiniferous tissues, although some preliminary correlations between structure and producing power have been obtained.

The number of streaks, or freshenings of the wound to allow exudation of oleoresin, varied slightly from year to year, being 33 in 1927, 26 in 1928, and 32 in 1929. The marked drop in the yield from both groups in 1928 is largely due to the fact that fewer streaks were cut, but particularly to the fact that the cups were not raised that year. As a result of not raising the cups, a greater proportion of the oleoresin was collected in the form of scrape in 1928 than in 1927, the virgin year, or in 1929, when the cups were raised. (Table 1.) Under these conditions considerable turpentine was undoubtedly lost by evaporation. There is also a greater opportunity for waste when a large proportion of the yield remains for the greater part of a season attached to the tree in the form of scrape, since fragments of scrape may break off of their own weight or be knocked off and lost or wasted on the ground.

SIGNIFICANCE OF RESULTS

This study shows that what started as an apparently insignificant winter grass fire in a longleaf pine stand caused serious damage. For the original 10 scorched trees studied the loss of about 50 per cent or more of the yield of oleoresin from the first working is largely traceable to the fire. If the total returns from the products of these trees are considered, a far greater loss in potential oleoresin and wood forming power results.

A reduction of growth in all dimensions which persisted for at least three years was found in the group of scorched trees as compared with the unscorched trees (*5*).

The microscopical examination of the material from the scorched and denuded longleaf pine trees that survived the fire gives tangible evidence of the native vigor of this species in recovering from fire as shown by its ability, however feeble, to build wood cells and to respond with high yields of oleoresin even when severely wounded by commercial turpentining. The ability of longleaf pines to adjust themselves to such adverse conditions should be considered in plans for planting and thinning.

SUMMARY

A winter fire, which killed the foliage but not the buds of a group of 10 young longleaf pines, resulted in a loss in the turpentined stand of over 50 per cent of the test trees, either by killing them outright or by causing them to cease to exude oleoresin.

The trees that survived the scorching showed a markedly reduced yield of oleoresin in the first year after the fire. They, however, recovered so that the yield of oleoresin per streak per tree increased during the next two years even under the exploitation of ordinary

commercial turpentining. During the second and third years after the fire the scorched trees that survived, yielded tree for tree practically as much oleoresin as unscorched trees.

The damage done by the scorching of the tops of the trees was plainly evident in the structure above the turpentined face in the injured trees, where the enfeebled wood formation was markedly less than the wood formation in the unscorched trees. This reduction consisted chiefly of missing annual-growth rings, poorly developed summer wood, and reduced oleoresin-yielding tissue. Yet these enfeebled trees, which survived the 1927 scorching and responded to commercial turpentining in 1928 and 1929 with a comparatively high yield of oleoresin, were able also to make an appreciable gain in wood formation in 1929, thus giving a striking demonstration of the potential energy and recuperative power of longleaf pine.

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SOME EFFECTS OF STRAW MULCH ON YIELD OF POTATOES[1]

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INTRODUCTION

It is well known that the potato (*Solanum tuberosum*) is a cool-climate crop. From a survey of the literature Bushnell (*3*)[2] concluded that the optimum temperature for the growth of the potato is near 64° F. In Ohio, mean July temperatures are much too high for its best development, ranging from 71° to 76° F. Any cultural treatment which would keep the soil cool during midsummer would therefore be expected to result in increased yields. Light-colored straw as mulch has occasionally been tried by potato growers, but it has not consistently given the anticipated benefits. Consequently mulching is not a widespread practice.

LITERATURE REVIEW

Brief reports from experiment stations indicate that straw mulch is more likely to produce increased yields of potatoes in the Central and Southern States than in the Northern. In Oklahoma, Waugh (*12*) and later Morris (*9*), reported increased yields from the use of straw mulch. In Ohio, Lazenby (*7*, *8*) and Alwood (*2*) obtained beneficial results with later varieties in one year out of three. In Illinois, Pieper, Burlison, and Flint (*10*) recently reported an average increase of 41.6 bushels per acre from an experiment extending through four seasons. They stated that straw is used to some extent in Illinois in small-scale potato production, and that it is especially beneficial in years of deficient moisture. In Nebraska, Emerson (*5*) obtained small increases with an early variety in two seasons and a decrease the third season. Recently, Werner (*14*), repeating the work in Nebraska, obtained increased yields in four seasons out of five. Farther north, Harwood (*6*) at Michigan and Whipple (*15*) at Montana found that straw mulch was detrimental, retarding growth and reducing yields.

Pieper, Burlison, and Flint (*10*) state that the culinary quality of the crop is much improved by mulching. Werner (*14*) found from a chemical analysis of the tubers of one crop that the starch content was considerably increased by the use of a straw mulch.

EXPERIMENTS AT WOOSTER

The possibility that straw mulch might prove a practical cultural practice in Ohio was brought to the attention of the writers in the course of a study primarily concerned with the lodging of wheat when sown after potatoes. In cultivated potato plots an excessive nitrate content developed which was deemed the cause of the frequent

[1] Received for publication Apr. 30, 1931; issued November, 1931.
[2] Reference is made by number (italic) to Literature Cited, p. 845.

lodging of the following crop of small grain (*13*). Straw was therefore applied as a mulch to some plots as a means of reducing the nitrate content.

On all plots, cut seed was planted with a machine planter to a depth of 2 to 3 inches; straw was applied immediately after planting, except where otherwise noted. Cultivated plots or those on which the application of straw was delayed were subjected to current farm practices with the aim of controlling weeds with minimum injury to the potatoes. Usually the plots were harrowed twice before the plants emerged; then a weeder of the Hallock type was used at intervals of a week or more until the plants branched. After a hard rain the weeder was ineffective, and a shallow cultivation was required to loosen the surface soil. After the plants branched, two or three shallow cultivations sufficed. The rows were not ridged or hilled, as level cultivation is the accepted practice in Ohio.

YIELD

On May 19, 1925, four plots, each one-fortieth acre, were planted to Russet Rural. Two of the plots were mulched immediately after planting at the rate of 10 tons of straw per acre. The layer was 8 to 10 inches deep when applied, and about 4 inches deep after settling. As shown in Table 1, the yields were approximately doubled by the mulch. The season, however, was peculiarly favorable for obtaining benefits from mulch. The mean temperatures for June and September, 1925, were far above normal, while the rainfall for the season was deficient, as shown in Table 2. Smith (*11*) has pointed out from correlations of weather and potato yields that when temperatures are above normal and rainfall is below normal there tends to be a reduction in the yield of potatoes in Ohio. Consequently, it might be anticipated that a straw mulch by correcting to some degree the unfavorable conditions would be particularly beneficial in such a season. The results were so striking as to suggest that the use of straw as a mulch might prove to be a practical procedure.

TABLE 1.—*Effect of using straw mulch on the yield of Russet Rural potato at Wooster, Ohio, 1925*

Plot No.	Treatment	Yield per acre
		Bushels
1	Straw mulch	277
2	Cultivated	131
3	Straw mulch	274
4	Cultivated	141

The experiment was repeated the two following seasons, with a modification to include a test of various quantities of straw.

TABLE 2.—*Mean temperature and rainfall and departures from normal for the summer months at Wooster, Ohio, 1925–1927*

[From records of C. A. Patton, Ohio Experiment Station]

Month	1925		1926		1927	
	Mean	Departure	Mean	Departure	Mean	Departure
	TEMPERATURE, °F.					
June	72.0	+4.3	64.4	−3.2	62.8	−4.7
July	70.4	−1.0	71.4	0	71.0	−.3
August	70.4	+.7	73.1	+3.4	65.6	−4.0
September	67.8	+3.9	65.2	+1.2	66.0	+2.0
	RAINFALL, INCHES					
June	2.24	−1.76	3.58	−0.41	3.36	−0.62
July	4.09	−.01	2.49	−1.57	4.28	+.22
August	1.88	−1.67	2.75	−.80	2.88	−.62
September	4.08	+.81	8.51	+5.11	2.69	−.69

In 1926, Russet Rurals were planted May 19, and the straw applied June 1. The yields, as shown in Table 3, were not as large from the mulched as from the cultivated plots. The detrimental effects of the mulch may have been due to abnormally heavy rainfall in September and October, which was injurious to potatoes on soils of the type used in the experiment (Wooster silt loam) and was probably more injurious under the straw where evaporation and run-off were impeded.

TABLE 3.—*Effect of using straw mulch on the yield of Russet Rural potato at Wooster, Ohio, 1926*

Plot No.	Treatment	Straw used per acre	Yield per acre
		Tons	*Bushels*
1	Straw mulch	4	163
2	Cultivated		200
3	Straw mulch	6	187
4	do	8	201
5	Cultivated		221
6	Straw mulch	10	208

In 1927, plots were planted on June 6 and mulched on June 14. The results, as reported in Table 4, were less consistent than were those of the preceding seasons, due largely to soil variation, but the highest yield was from the plot with the heaviest straw mulch. It was noted in both seasons that annual weeds readily penetrated the straw when less than 8 tons per acre were used.

TABLE 4.—*Effect of using straw mulch on the yield of Russet Rural potato at Wooster, Ohio, 1927*

Plot No.	Treatment	Straw used per acre	Yield per acre
		Tons	*Bushels*
1	Cultivated		133
2	Straw mulch	4	162
3	do	6	164
4	Cultivated		181
5	Straw mulch	8	177
6	do	10	209
7	Cultivated		153

Average increases in yield for the three seasons due to the application of 8 or 10 tons of straw per acre were calculated by comparing the yield of individual plots with that of adjacent cultivated plots.

The average increase of 50 bushels per acre, as shown in Table 5, might by itself be a basis for recommending the use of straw mulch as a farm practice, but the variation from season to season was too great to permit predictions as to the probable value of the practice over a longer period.

TABLE 5.—*Average increase or decrease in yield of Russet Rural potato when straw mulch was applied at the rate of 8 and 10 tons per acre, Wooster, Ohio*

[Data summarized from preceding tables]

Season	Average yield of cultivated (unmulched) plots	Average yield of mulched plots	Increase or decrease
	Bushels	*Bushels*	*Bushels*
1925	136	275.5	139.5
1926	221	204.5	−16.5
1927	167	193	26
Average	174.66	224.33	49.7

QUALITY

In occasional seasons the weather is so unfavorable in midsummer at Wooster that growth of tubers nearly ceases, they lose their capacity to develop normally, and when favorable growth conditions ensue knobby outgrowths develop. The most common type of outgrowth is a large knob at the apical bud end; such malformed tubers are not

FIGURE 1.—A, Tubers with secondary apical knobs, from cultivated plot, 1926; B, normal tubers from mulched plot. Photographed in March, at which time the "dumb-bell" tubers were noticeably wilted at the stem end

inappropriately called "dumb-bells." The effectiveness of straw mulch in preventing this abnormality was conspicuous in 1926. Typical tubers from the unmulched and mulched plots are shown in Figure 1. The photograph was taken in March, 1927, at which time the stem end of the dumb-bells was shriveling, while the tubers from the strawed plot remained firm.

The following season no abnormal shapes appeared, but the potatoes from the cultivated plots wilted and sprouted earlier in storage.

SOIL TEMPERATURES

The temperature of the unmulched and mulched plots was determined in 1925 by a Friez double soil thermograph, the bulbs of which

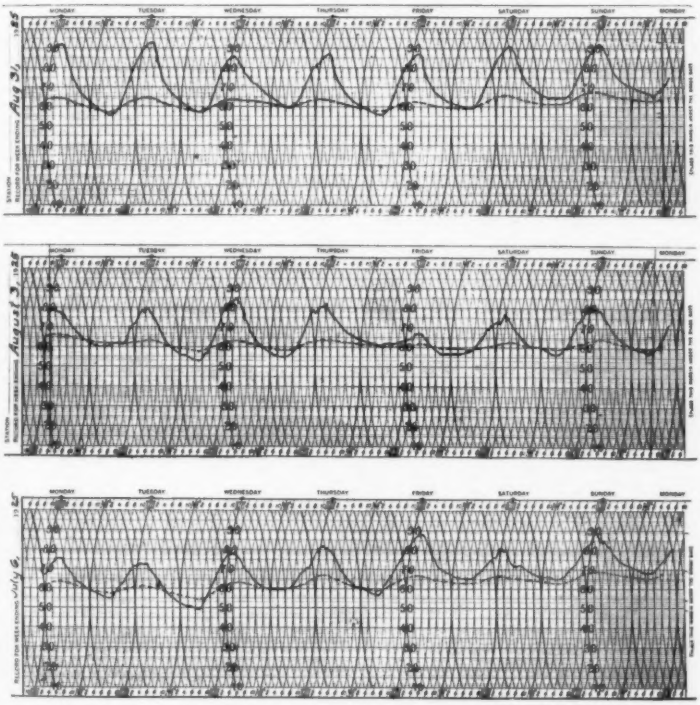

FIGURE 2.—Typical thermograph records of soil temperatures; the solid line records the temperature of the cultivated soil at a depth of 1 inch, the broken line that of the mulched soil

were buried in the soil to a depth of 1 inch. The thermograph was placed in position June 22 and continuous records were obtained until harvest, September 29. The average mean temperature for the whole period was 68.8° F. for the cultivated and 63.3° for the mulched plots. The difference during the heat of the day was much more marked, as shown in typical record sheets, Figure 2. The extreme difference in soil temperature occurred in August, when it amounted to 29 degrees. Differences of 25 degrees in the middle of the day were quite common.

SOIL MOISTURE AND SOIL NITRATES[1]

Moisture determinations were made on composite soil samples at intervals of about 10 days during 1925. As shown in Figure 3, the straw mulch was effective in maintaining a higher water content in the soil.

Nitrate determinations, made at the same time as the moisture determinations, disclosed a somewhat reduced nitrate content of the soil under the straw. (Fig. 4.)

EXPERIMENTS WITH EARLY POTATOES IN SOUTHERN OHIO

From the results obtained at Wooster it appeared that the use of straw mulch might be more consistently beneficial on soils which drained more rapidly and which were farther south where summer temperatures are higher. Experiments were therefore started at the Hamilton County experiment farm, near Cincinnati. In this district the mean summer temperature is more than 5 degrees higher than at Wooster, ordinarily precluding successful production of late varieties of potatoes. Early maturing varieties are therefore grown. But even when these are planted as early as possible they encounter the hot weather of July before maturity. That the potato is particularly sensitive to high temperature during the period of tuber growth has been emphasized by Bushnell (*4*). It therefore seemed reasonable to expect that a straw mulch would prove beneficial to the early crop in southern Ohio.

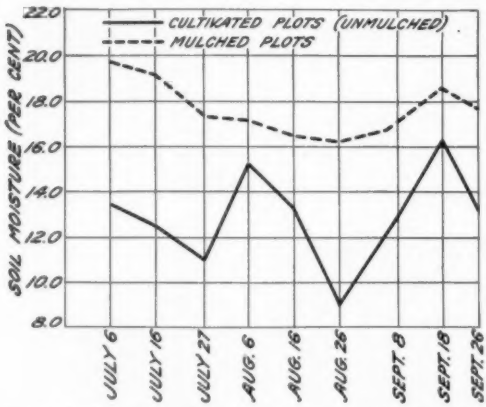

FIGURE 3.—Soil-moisture determinations of cultivated and mulched plots

In actual farm experience, however, the expected benefits have not always been obtained. An explanation of this may be found in Emerson's (*5*) work at Nebraska. Emerson concluded from one season's data that it was advisable to delay application of the straw until the soil was warm because a mulch applied at the time of planting retarded early growth.

To obtain further information on this point, the straw mulch was applied in the Hamilton County experiments on three different dates: (1) Immediately after planting in late March; (2) when the plants were up in late April; and (3) after the soil had reached a temperature of about 65° F. in late May or early June.

[1] Soil moisture and nitrates were determined by V. H. Morris.

The applications were made in triplicate, the plots being 0.027 acre each. A 4–10–6 fertilizer at the rate of 1,000 pounds per acre was used throughout. The straw was applied at the rate of 10 tons per acre. In 1928, the Early Ohio variety was used; in 1929, the Irish Cobbler. To facilitate digging it was found advisable to remove most of the straw before harvesting. Any detrimental effects which might have ensued from plowing under large amounts of straw were thus avoided.

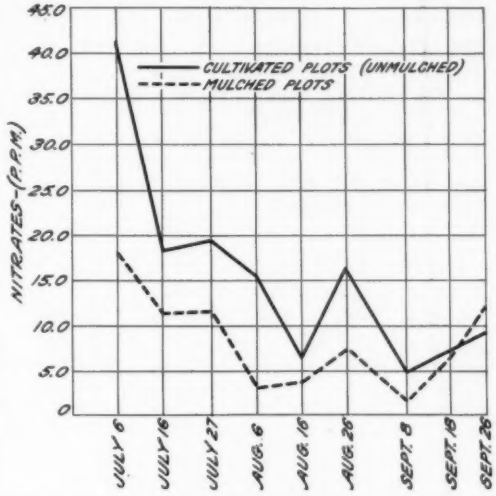

FIGURE 4.—Soil-nitrate determinations of cultivated and mulched plots

The yields for both seasons are summarized in Table 6. The odds of significance for the increases over cultivated plots were calculated from unpublished tables of M. T. Myers derived from those of Student (*1*).

TABLE 6.—*Effect of using straw mulch applied at three different dates, on the yield of early potatoes, Hamilton County, Ohio, 1928 and 1929*

[Yield of marketable potatoes and increase over cultivated plots in bushels per acre]

Treatment	Variety, date planted, yield, and increase or decrease compared with yields from unmulched plots					
	Early Ohio, Mar. 28, 1928			Irish Cobbler, Mar. 25, 1929		
	Yield[a]	Increase or decrease	Odds	Yield[b]	Increase	Odds
Mulched when planted, about Apr. 1	121	−14	11 : 1	323	48	36 : 1
Mulched early in May	165	+30	17 : 1	366	91	26 : 1
Mulched early in June	148	+13	7 : 1	334	59	33 : 1

[a] Average yield of three cultivated, unmulched plots, 135 bushels per acre.
[b] Average yield of three cultivated, unmulched plots, 275 bushels per acre.

The first mulch, applied immediately after planting, retarded early growth in both seasons and the plants showed typical symptoms of nitrogen deficiency. In 1928 this early mulch reduced the yield below that of the cultivated plots.

The second mulch, applied after the plants were up, gave the highest yields in both seasons. In appearance the plants on these plots were the best, and they remained green for more than a week after the cultivated plants had died prematurely from midsummer heat

When the last mulch was applied some of the leaves were covered and some were unavoidably broken, which may in part account for the fact that the yield from this mulch was lower than from the preceding one. But it is also probable that the earlier application was beneficial during the period in May when the late-mulched plots were still being cultivated.

The mulches were conspicuously beneficial in 1929 when the weather was peculiarly favorable for potatoes. Even though the mean temperature was below normal and the rainfall was abundant, resulting in high yields on cultivated plots, the mulch increased yields, a result quite different from that obtained at Wooster in a comparable season.

DISCUSSION

Although the value of straw mulch as a means of reducing temperature has been the primary consideration in this work, no direct evidence has been secured to show that the reduction in temperature was more important than the conservation of soil moisture. The suggestion that temperature is the more important factor in Ohio is based upon the correlations of weather and potato yields compiled by Smith (*11*) and the observations enumerated by Bushnell (*4*). In a 3-year series of tests conducted by Werner at Lincoln, Nebr. (*14*), in which he made a comparative study of the effect of irrigation and straw mulch on yield of potatoes, he found that under the conditions of his experiments benefits were due both to conservation of moisture and to reduction of temperature. Irrigation increased the yields of potatoes for three successive seasons, and so also did straw mulch. With both irrigation and straw mulch, however, the yields were further increased. Presumably Werner added an abundance of water to the cultivated plots, so that the additional increase from the straw may be attributed to its effect on the temperature.

Practical benefits from straw mulch then would be expected in regions where the temperature is above the optimum for the growth of the potato and when moisture is deficient. An important practical conclusion from the experiments in southern Ohio is that detrimental effects may accrue if the mulch is applied too early, and that to obtain the best results with early varieties the application should be delayed until after the plants are up.

SUMMARY

When straw mulch was applied to the Russet Rural potato, a main crop variety, at Wooster, Ohio, the yield was twice as great as that from cultivated plots in one season, but there was no marked effect on yield in the two following seasons.

In southwestern Ohio, straw mulch applied to early potatoes after the plants were up increased the yield 31 and 91 bushels per acre, respectively, in two seasons. A similar mulch applied at the time of planting retarded early growth and hence was not so beneficial.

When the straw was applied at the rate of less than 8 tons per acre, annual weeds readily penetrated it.

The straw mulch reduced the soil temperature below that of cultivated plots, conserved the moisture content, and depressed the nitrates.

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